Attorney Docket No.: 02307V-142800US
Client Reference No.: B04-048

PATENT APPLICATION

PEPTIDE BETA-STRAND MIMICS BASED ON PYRIDINONES, PYRAZINONES, PYRIDAZINONES, AND TRIAZINONES

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AS FILED IN THE U.S.P.T.O. -- February 24, 2004

PEPTIDE BETA-STRAND MIMICS BASED ON PYRIDINONES, PYRAZINONES, PYRIDAZINONES, AND TRIAZINONES

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0001] This invention was made with government support under Grant No. GM-30759 awarded by the National Institutes of Health. The Government has certain rights to this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] This invention resides in the field of proteins and the complexations and interactions of proteins with other proteins and with nucleic acids through β-sheet interactions. The particular areas addressed by this invention are compositions for and methods of modifying the ability of proteins to enter into these interactions and the various benefits that are derived from such modifications, including changes to the biological activity of the proteins.

1. Description of the Prior Art

[0003] The conformation of proteins and peptides is largely governed by secondary structural elements, such as α-helices, β-turns, and β-strands, which determine the three-dimensional orientation of the amino acid side chains and thereby the longer range interstrand and intermolecular interactions. Both β-strands and the β-sheets derived from β-strands play important roles in protein-protein interactions as well as the association of proteins with other biopolymers such as ribosomal RNA and nucleic acids. Disclosures of these roles are found in Fitzgerald, F.M.D., et al., *J. Biol. Chem.* **1990**, vol. *265*, 14209; Zutshi, R., et al., *J. Am. Chem. Soc.* **1997**, vol. *119*, 4841; Babe, L. M., et al., *Protein Sci.* **1992**, vol. *1*, 1244; Siligardi, G., et al., *Biopolymers (peptide science)* **1995**, vol. *37*, 281; Buckle, A. M., et al., *Proc. Natl. Acad. Sci. USA* **1997**, vol. *94*, 3571; Taneja, B. C., et al., *Protein Engineering* **1999**, vol. *12*, 815; Stern, L. J., et al., *Nature* **1994**, vol. *368*, 215; Moss, N., et al., *J. Med. Chem.* **1996**, vol. *39*, 2178; Sauer, F. G., et al., *Science* **1999**, vol. *285*, 1058; Karlsson, K. F.,

et al., *J. Bioorg. Med. Chem.* **1998**, vol. *6*, 2085; Maitra, S.; Nowick, J. S. In *The Amide Linkage*; Greenberg, A.; Breneman, C. M.; Liebman, J. F.; Eds.; John Wiley & Sons, Inc.: New York, NY, **2000**; pp 495-518; Lynn, D. G., et al., *J. Struc. Biol.* **2000**, vol. *130*, 153; Wilkinson, A. J. *Chem. Biol.* **1996**, vol. *3*, 519; Jones, S., Thornton, J. M. *Proc. Natl. Acad. Sci. USA* **1996**, vol. *93*, 13; Tateno, M., et al., *Biopolymers* **1998**, vol. *44*, 335; Connolly, K. M., et al., *J. Mol. Biol.* **2000**, vol. *300*, 84; Cohen, S. X., et al., *EMBO J.* **2003**, vol. *22*, 1835. For example, the β-sheet-like association and precipitation of hydrophobic protein fragments in amyloid plaques is strongly implicated in neurodegenerative diseases, as disclosed by Cohen, F. E.; Kelly, J. W. *Nature* **2003**, vol. *426*, 905; Roloff, E. V., et al., *Cell. Mol. Life Sci.* **1999**, vol. *55*, 601; Yatin, S. M., et al., *J. Mol. Neurosci.* **1998**, vol. *11*, 183; and Prusiner, S. B., et al., *Cell* **1998**, vol. *93*, 337. Furthermore, various biological processes depend on the accessibility of individual peptide strands. Examples of these processes are:

vancomycin complexation of the Lys-D-Ala-D-Ala peptide in bacterial cell wall synthesis;

homodimerization of HIV protease, which involves a "fireman's grip" β-sheet interaction among the N-terminal residues;

heterodimerization of ribonucleotide reductase and HIV reverse transcriptase, which can be blocked with soluble oligopeptides corresponding to part of the interface regions;

dimerization of the λ-Cro repressor via an antiparallel β-strand; and

protein-protein association via PDZ domains.

[0004] Systems that mimic and block these interactions are disclosed by Smith, A. B., et al., *J. Am. Chem. Soc.* **1992**, vol. *114*, 10672; Smith, A. B., et al., *J. Am. Chem. Soc.* **1994**, vol. *116*, 9947-9962; Smith, A. B., et al., *J. Am. Chem. Soc.* **1995**, vol. *117*, 11113-11123; Smith, A. B., et al., *Bioorg. Med. Chem.* **1996**, vol. *4*, 1021; Smith, A. B., et al., *J. Am. Chem. Soc.* **1999**, vol. *121*, 9286-9298; Smith, A. B., et al., *Organic Letters* **2000**, vol. *2*, 2037; Smith, A. B., et al., *Organic Letters* **2000**, vol. *2*, 2041; Hirschmann, R., et al, United States Patent No. 5,489,692, issued February 6, 1996; Hirschmann, R.F., et al, United States Patent No. 5,514,814, issued May 7, 1996; Hirschmann, R.F., et al, United States Patent No. 5,770,732, issued June 23, 1998; Smith, III, A.B., et al, United States Patent No. 6,034,247, issued March 7, 2000; Nowick, J.S., et al., *J. Am. Chem. Soc.* **2000**, vol. *122*, 654-661; Nowick, J.S., et al., *J. Am. Chem. Soc.* **2001**, vol. *123*, 5176-5180; Nowick, J., et al., International Patent Application No. WO 01/14412, published March 1, 2001, under the

Patent Cooperation Treaty; Nowick, J. S., Chung, D. M. *Angew. Chem. Int. Ed.* **2003**, vol. *42*, 1765; Boumendjel, A., Roberts, J. C., Hu, E., Pallai, P. V., Rebek, J., *J. Org. Chem.* **1996**, vol. *61*, 4434; and Kemp, D.S., et al., *J. Org. Chem.* **1990**, vol. *55*, 4650-7.

[0005] The effectiveness and utility of these mimicking systems is enhanced by incorporating sequence selectivity into the structures. Sequence-selective peptide complexing agents that interact with extended peptides or with exposed loops on proteins offer potential as biological tools and therapeutic approaches, as well as serving as the basis for a proteomics equivalent of gene expression analysis.

[0006] Reported investigations of sequence-selective peptide complexing agents range from non-peptidic cavitands disclosed by Still, W.C., *Acc. Chem. Res.* **1996**, vol. 29, 155-163, Chen, C.-T., Wagner, H., Still, W. C., *Science* **1997**, vol. *279*, 851; Malteic, M., et al., *Angew. Chem. Int. Ed.* **1996**, vol. *35*, 1490, to highly flexible structures in which two peptide or peptide-like strands are connected by a variety of linkers, reported for example by LaBrenz, S.R., et al., *J. Am. Chem. Soc.* **1995**, vol. 117, 1655-1656; Löwik, D.W.P.M., et al., *Angew. Chem., Int. Ed.* **1998**, vol. 37, 1846-1850; Hioki, H., et al., *Tetrahedron Lett.* **1999**, vol. 40, 6821-6825; Botana, E., et al., *Chem. Commun.* **2001**, 1358; Braxmeier, T., et al., *Chem. Eur. J.* **2001**, vol. *7*, 1889; Davies, M., et al., *J. Org. Chem.* **1998**, vol. *63*, 8696; Fessmann, T., et al. *Angew. Chem., Internat. Edit. Engl.* **1999**, vol. *38*, 1993; Wennemers, H., et al., *Chem. Eur. J.* **2001**, vol. *7*, 3342; Bouras, A., et al., *J. Med. Chem.* **1999**, vol. *42*, 957; Song, M., et al., *Bioorg. Med. Chem. Lett.* **2001**, vol. *11*, 2465; Boyce, R., et al., *J. Am. Chem. Soc.* **1994**, vol. *116*, 7955; Cheng, Y., Senaga, T., Still, W.C., *J. Am. Chem. Soc.* **1996**, vol. *118*, 1813; Ghosh, I., Chmielewski, *J. Chemistry & Biology* **1998**,vol. *5*, 439; and Zutshi, R.; Chmielewski, *J. Bioorg. Med. Chem. Lett.* **2000**, vol. *10*, 1901. The host-guest complex produced by these highly flexible structures is a three-stranded β-sheet whose outer strands provide both affinity through the hydrogen-bonding network and selectivity through interactions between side chains. The success of this approach has been limited however for several reasons. One reason is that the design fails to include bridging units that provide precise positioning of the side arms. Another is that the high flexibility of the peptide arms causes formation of the desired complex to be disfavored entropically. A third is that hydrogen bonding, while favorable in organic solvents, is not a strong binding force in water in the absence of pre-organization. For those host-guest pairs that have sufficient hydrophobic binding to assemble in water, the high degree of hydrophobic binding tends to cause them to aggregate further.

[0007] All published materials cited throughout this specification are hereby incorporated herein by reference.

SUMMARY OF THE INVENTION

[0008] It has now been discovered that 1,6-dihydro-3(2*H*)-pyridinones that are substituted in the 6-position (designated the "α-position") with an amino acid side chain are highly effective when used as units in peptide β-strand mimics, and that related heterocycles whose 2- and 4-position ring vertices are replaced by additional N atoms (or NH groups), including those that are substituted with an amino acid side chain at the α-position, are also highly effective as units in peptide β-strand mimics. Thus, four classes of structures are disclosed herein as amino acid substitutes in peptide analogs. These four classes are:

(1) α-substituted (i.e., 6-substituted) 1,6-dihydro-3(2*H*)-pyridinones,

(2) 4,5-dihydro-2(3*H*)-pyrazinones and α-substituted (i.e., 5-substituted) 4,5-dihydro-2(3*H*)-pyrazinones,

(3) 1,6-dihydro-3(2*H*)-pyridazinones and α-substituted (i.e., 6-substituted) 1,6-dihydro-3(2*H*)-pyridazinones, and

(4) 1,6-dihydro-1,2,4-triazin 3(2*H*)-ones and α-substituted (i.e., 6-substituted) 1,6-dihydro-1,2,4-triazin 3(2*H*)-ones,

in which the α-substituent, in those species where such a substituent is indicated, is an amino acid side chain. Collectively, these four classes are referred to herein as "azacyclohexenones," the prefix "aza" denoting either one, two, or three nitrogen atoms. The term "azacyclohexenones" is used herein to encompass both those species that are unsubstituted at the α-position and those that bear an amino acid side chain at the α-position, the latter subset being referred to occasionally herein as "α-substituted azacyclohexenones." Peptide analogs in which at least one but less than all amino acids is replaced by an azacyclohexenone unit or an α-substituted azacyclohexenone unit of the present invention readily enter into β-sheet-like interactions of particularly high strength, and those that are α-substituted enter into β-sheet-like interactions that are even more sequence-specific due to the amino acid side chain(s) at the α-position of the unit(s). The present invention also extends to peptide analogs in which one or more amino acids are replaced by any one of the azacyclohexenones described above and one or more additional amino acids are replaced by

1,6-dihydro-3(2*H*)-pyridinones that bear no α-substituent, such as those disclosed in United States Published Patent Application No. US 2003-0073721 A1, published April 17, 2003.

[0009] Also encompassed by this invention are hybrid constructs that consist of a peptide analog sequence covalently linked to another peptide analog sequence or to a conventional peptide sequence. In the peptide analog sequences, at least one but not all amino acids is replaced by an azacyclohexenone unit of the present invention. The linkage between the two sequences may consist of amino acid units that connect the sequences in a head-to-tail orientation and that permit a β-turn, in which case the peptide analog sequence, by virtue of its strong tendency to enter into stable β-sheet-like interactions with conventional peptide and other peptide analog sequences, forms a β-hairpin that stabilizes the other sequence in an extended β-strand conformation. The linkage between the two sequences may consist of other connecting units than amino acids. Moreover, the linkage may connect the sequences in a head-to-head or a tail-to-tail orientation so that the resulting molecule can form a complex with a separate conventional amino acid sequence. Examples of such linkages are found in the prior art related to sequence-selective peptide complexation (cited above). The present invention is not however limited to those specific linkages.

[0010] The peptide analogs and peptide-analog hybrids of this invention have many applications. They can serve for example as tools for studying β-sheet nucleation, propagation, and suppression. They can also serve as prophylactic or palliative agents in physiological conditions that involve or are controllable by β-sheet interactions. For example, these peptide analogs and hybrids can be used in the treatment of prion diseases such as "mad cow disease" and other neurodegenerative diseases such as Alzheimer's disease which arise from the association of certain hydrophobic proteins to form insoluble β-sheet aggregates known as amyloid complexes. This utility arises from the enhanced ability of the analogs and hybrids of this invention to bind to an exposed surface of the amyloid β-sheet complex and prevent further aggregation. The peptide analogs and hybrids can also be used for blocking the infectivity of the human immunodeficiency virus by inhibiting the association of the viral gp 120 protein with the CD4 receptor on the T-lymphocyte cell surface. A still further use is the blocking of the effects of inflammatory chemokines that are involved in allergic reactions, psoriasis, arthritis, atherosclerosis, multiple sclerosis, and cancer.

[0011] Peptide analogs in accordance with this invention function similarly to antibodies by binding to peptides and proteins in a sequence-selective manner. As such, the peptide analogs and peptide-analog hybrids of this invention are useful for example as protein purification media in affinity chromatography. They are also useful as components in diagnostic devices or kits, where they can be used for the concentration and identification of peptide and protein analytes. This antibody-type character also provides utility *in vivo*, where the peptide analogs and peptide-analog hybrids can be used for therapeutic effects by forming complexes with and blocking the action of specific peptide hormones or by targeting attached radiopharmaceuticals or cytotoxic agents to specific sites in the body. A collection of peptide analogs and hybrids in accordance with this invention can be arranged in an array such as that of a proteomics chip for use in an assay for the levels of expression of specific proteins in different tissues and under different conditions. Other uses will be readily apparent to those skilled in the art.

[0012] The present invention thus resides in:

Azacyclohexenones in which the prefix "aza" denotes that one, two, or three carbon atoms in the cyclohexenone ring are replaced by a nitrogen atom, including α-substituted azacyclohexenones in which "α-substituted" denotes that the ring carbon opposite to the carbonyl group is substituted with an amino acid side chain, the azacyclohexenones, α-substituted or otherwise, being either functionalized for linkage to each other or to amino acids by formation of carbon-nitrogen bonds, or covalently bonded to one or more amino acids through carbon-nitrogen bonds

Peptide analogs in which at least one amino acid, but not all, is replaced by an azacyclohexenone group, α-substituted or otherwise, as described above

Peptide-analog hybrids consisting of peptide analogs covalently linked to other peptide analogs or to conventional peptides as described above, in head-to-tail, head-to-head, or tail-to-tail orientation

The use of peptide analogs and peptide-analog hybrids as described above for inhibiting β-sheet-like interactions between proteins

The use of peptide analogs and peptide-analog hybrids as described above for inhibiting the biological activity of a peptide or protein

The use of peptide analogs and peptide-analog hybrids as described above for extracting a target peptide from a mixture of peptides or a target protein from a mixture of proteins

[0013] Other structures, uses, embodiments, applications and features of the invention will be apparent from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. **1** is an illustrative reaction scheme for one subclass of constructs within the scope of this invention.

[0015] FIG. **2** is an illustrative reaction scheme for a second subclass of constructs within the scope of this invention.

[0016] FIG. **3** is an illustrative reaction scheme for a third subclass of constructs within the scope of this invention.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

Definitions

[0017] The term "β-strand conformation" is used herein to denote the three-dimensional conformation of a single peptide strand in which the strand is elongated such that its amide groups form a planar zig-zag backbone and the amino acid side chains extend out of the plane to either or both sides. A peptide strand may assume this conformation either on its own or in combination with another peptide (or peptide analog) in a *β*-sheet-like interaction as defined below.

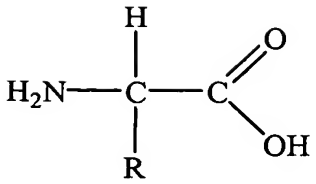
[0018] The term "*β*-sheet-like interaction" is used herein to denote the interaction between two peptides both of which are in a *β*-strand conformation, in which the two strands are held together side-by-side with hydrogen bonding between carbonyl oxygens in one backbone and amide NH groups in the other (and vice versa). The orientation of the strands may be parallel or anti-parallel, although the anti-parallel relationship is preferred. The term also extends to the analogous interaction that occurs when one of the peptides is replaced by a peptide analog or another elongated molecule in which similar hydrogen bonds are formed along the lengths of the molecules. Any peptide analog in accordance with this invention may thus enter into a

β-sheet-like interaction with a peptide, with itself, or with another peptide analog. An individual peptide may engage in β-sheet-like interactions with two such peptides, analogs or other molecules, one on each side of the first peptide.

[0019] The term "β-turn" is used herein to denote a sharp 180-degree ("hair-pin") turn in a peptide sequence that places the segments on either side of the turn in sufficient proximity to engage in hydrogen bonding between opposing units in the segments such that the segments align to form a β-sheet-like interaction. In recitations of a linkage that "permits ... a β-sheet-like interaction," "permits a hair-pin turn," and similar phrases, the word "permit" denotes that the linkage is capable of adopting a β-turn conformation with little or no resistance, as opposed to linkages that offer steric or electronic resistance to adopting a β-turn conformation.

[0020] The term "peptide" is used herein to denote a compound containing two or more of amino acid residues joined by an amide bond formed from the carboxyl group of one residue and the amino group of the adjacent residue. The term "amino acid" includes both naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics whose properties are similar to those of the naturally occurring amino acids. Naturally occurring amino acids are those that are encoded by the genetic code, as well as those that are modified after expression, such as hydroxyproline, carboxyglutamate, O-phosphoserine, and glycosylated amino acids. Amino acid analogs are compounds having functionalities similar to those of naturally occurring amino acids, i.e., an amino group, a carboxyl group, and one or more side chains attached to a framework of from 1 to 4 carbon atoms. Many such analogs are known to those skilled in the art, including but not limited to homoserine, norleucine, methionine sulfoximine, phenylglycine, (p-fluorophenyl)alanine, β-alanine, α-aminoisobutyric acid, *tert*-leucine, and β-methylaspartic acid. The term "amino acid mimetic" is used herein to denote a moiety other than a naturally occurring amino acid that conformationally and functionally serves as a substitute for an amino acid in a peptide while still allowing the peptide to enter into β-sheet interactions. A variety of amino acid mimetics is listed by Morgan, B.A., and J.A. Gainor, *Ann. Rep. Med. Chem.*, vol. 24, 243-252 (1989). The term "peptide" includes, but is not limited to, proteins.

[0021] The term "amino acid side chain" denotes the group represented by the "R" in the amino acid formula



and includes any of the side chains in naturally occurring amino acids as well as those in modified amino acids and amino acid mimetics, and includes side chains in protected as well as unprotected form. While glycine is an amino acid having an H atom in the R position, the term "amino acid side chain" as used herein does not include H. In general, preferred amino acid side chains are C_1-C_6 alkyl, C_1-C_6 alkyl interrupted by -O-, C_1-C_6 alkyl interrupted by -S-, hydroxy-(C_1-C_6 alkyl), carboxy-(C_1-C_6 alkyl), amino-(C_1-C_6 alkyl), guanidino-(C_1-C_6 alkyl), carbamoyl-(C_1-C_6 alkyl), mercapto-(C_1-C_6 alkyl), indolyl-(C_1-C_3 alkyl), phenyl-(C_1-C_3 alkyl), hydroxyphenyl-(C_1-C_6 alkyl), halophenyl-(C_1-C_6 alkyl), imidazolyl-(C_1-C_6 alkyl), phenyl, and sulfoximino-(C_1-C_6 alkyl). The most preferred are C_1-C_4 alkyl, hydroxy-(C_1-C_2 alkyl), carboxy-(C_1-C_2 alkyl), amino-(C_3-C_5 alkyl), guanidino-(C_2-C_4 alkyl), carbamoyl-(C_1-C_2 alkyl), mercapto-(C_1-C_2 alkyl), methylthio-(C_1-C_3 alkyl), indolylmethyl, phenyl-(C_1-C_2 alkyl), and hydroxyphenyl-(C_1-C_2 alkyl).

[0022] The term "activated leaving group" is used herein to denote a radical or group of atoms that is displaced from a carbon atom by the attack of a nucleophile in a nucleophilic substitution reaction.

[0023] The term "protecting group" is used herein to denote a radical or group of atoms that is bound to a particular atom of a molecule to prevent that atom from participating in reactions occurring on other portions of the molecule.

[0024] The term "amine protecting group" is used herein to denote a radical or group of atoms that is bound to an amine nitrogen atom of a molecule to prevent that nitrogen atom from participating in reactions occurring on other portions of the molecule. The term "amine-protected" denotes the structural characteristic of a molecule containing an amine nitrogen atom by which that nitrogen atom is prevented from participating in reactions occurring on other portions of the molecule.

[0025] The term "carboxy protecting group" is used herein to denote a radical or group of atoms that is bound to a carboxy oxygen atom of a molecule to prevent that oxygen atom from participating in subsequent reactions occurring on other portions of the molecule. The term "carboxy-protected" denotes the structural characteristic of a molecule containing a

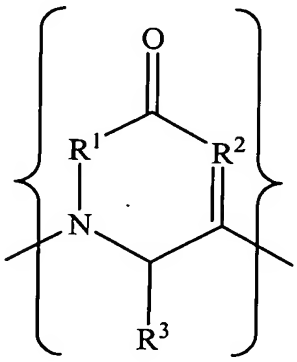
carboxy oxygen atom by which that oxygen atom is prevented from participating in reactions occurring on other portions of the molecule.

[0026] The term "side chain protecting group" is used herein to denote a radical or group of atoms that is bound to a nitrogen atom or an oxygen atom or a sulfur atom contained within an amino acid side chain or within the α-substituent of an azacyclohexenone to prevent that side chain functionality from participating in reactions occurring on other portions of that molecule. The term "side chain-protected" denotes the structural characteristic of a molecule containing a side chain functionality by which that side chain functionality is prevented from participating in reactions occurring on other portions of the molecule.

[0027] The term "solid support" is used herein to denote any inert solid that can be used to facilitate the separation of bound species from free species in the course of a solid-supported synthesis or in a binding interaction such as a chromatographic separation or any of various analytical procedures that involve affinity-type binding. Solid supports include particles such as those used in chromatography columns as well as the inner walls of reaction vessels such as test tubes and the wells of microtiter plates, and other configurations well known to clinicians and laboratory technicians. Examples of materials used as solid supports are agarose, polystyrene, polyacrylamide, and these materials modified by poly(ethylene glycol). A peptide analog can be attached to these supports through the C-terminus (for example by an ester or amide linkage), through the N-terminus (for example, by a urea or carbamate linkage), or through a functionalized side chain (for example, by ester, amide, urea, carbamate, disulfide, or ether linkages).

Compounds, Peptide Analogs and Constructs of the Invention

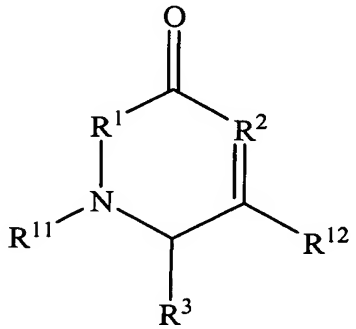
[0028] The generic formula for the azacyclohexenone unit included in the compounds, analogs, and constructs of this invention, is the structure shown between in the brackets of the following formula:



[0029] In this formula, R^1 is CH_2 or NH, R^2 is CH or N, and for those structures in which R^1 is CH_2 and R^2 is CH, R^3 is an amino acid side chain, while for those structures in which R^1 is NH, or R^2 is N, or both R^1 is NH and R^2 is N, R^3 is either H or an amino acid side chain. When two or more of these units are present in a peptide analog, hybrid, or other molecular structure in accordance with this invention, R^1, R^2, and R^3 of any one of these units in the structure are either the same as or different from R^1, R^2, and R^3 of any other of these units in the structure. Of the four structurally distinct classes of these units recited above, 1,6-dihydro-3(2*H*)-pyridinones are those in which R^1 is CH_2 and R^2 is CH, 4,5-dihydro-2(3*H*)-pyrazinones are those in which R^1 is CH_2 and R^2 is N, 1,6-dihydro-3(2*H*)-pyridazinones are those in which R^1 is NH and R^2 is CH, 1,6-dihydro-1,2,4-triazin 3(2*H*)-ones are those in which R^1 is NH and R^2 is N, and those that are α-substituted bear an amino acid side chain (as defined above to mean substituents other than H itself) at the 6-position in 1,6-dihydro-3(2*H*)-pyridinones, 1,6-dihydro-3(2*H*)-pyridazinones, and 1,6-dihydro-1,2,4-triazin 3(2*H*)-ones, and at the 5-position in 4,5-dihydro-2(3*H*)-pyrazinones. The pyrazinones, i.e. are those in which R^1 is CH_2 and R^2 is N, substituted or unsubstituted at the α-position, are preferred.

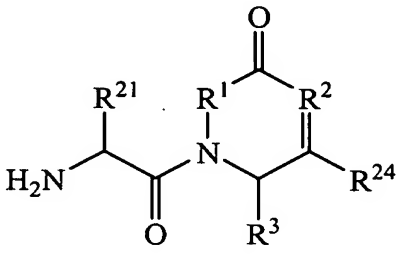
[0030] The α-substituted azacyclohexenone derivatives (i.e., those in which R^3 is an amino acid side chain) exist in two stereoisomeric forms. The enantiomers of the α-substituted azacyclohexenones that correspond to the L configuration of the natural amino acids are the more desirable isomers because peptide analogs incorporating these enantiomers enter into β-sheet-like interactions with natural peptides and proteins more readily and with higher affinity and selectivity than peptide analogs incorporating α-substituted azacyclohexenones of the opposite configuration.

[0031] The functionalized azacyclohexenones of this invention are those having the formula



in which R^{11} is a nitrogen protecting group, and R^{12} is either OH, SH, or an activated leaving group. As those skilled in the art will recognize, these structures are capable of other tautomeric forms. Such tautomeric forms are likewise within the scope of this invention.

[0032] Peptide analogs of this invention include compounds of the following formulas:



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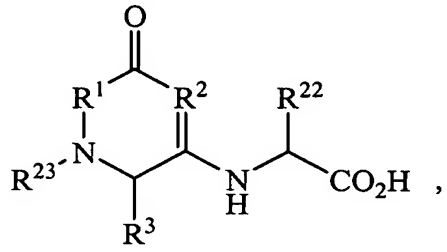

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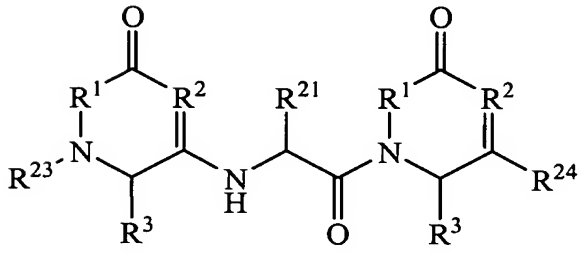

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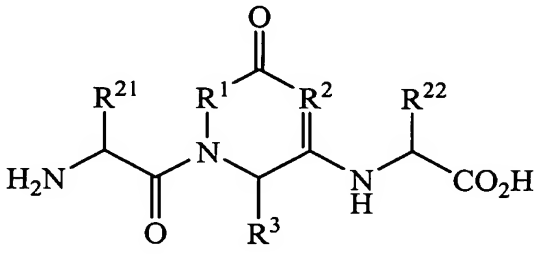


in which:

R^{21} is H or an amino acid side chain,

R^{22} is H or an amino acid side chain and may be the same as or different from R^{21} when both appear in the same compound,

R^{23} is either H or an amine protecting group, and

R^{24} is either:

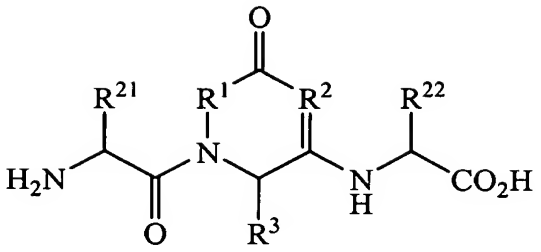
an activated leaving group,

OR^{25} where R^{25} is H or an oxygen-protecting group,

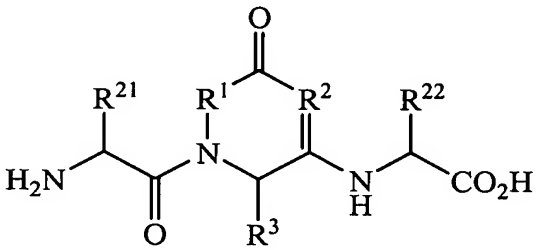
SR^{26} where R^{26} is H or an alkyl or aryl group, or

$N(R^{27})_2$ where the R^{27}'s are independently H, alkyl, or aryl.

[0033] Also included are amine-protected analogs of the compounds that terminate in H_2N-, carboxy-protected analogs of the compounds that terminate in -CO_2H, carboxy-activated analogs of the compounds that terminate in -CO_2H, amine-protected and carboxy-protected analogs of

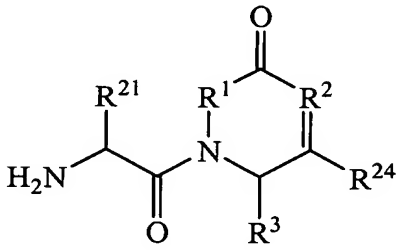


and amine-protected and carboxy-activated analogs of



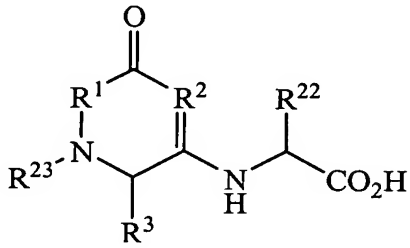
When two azacyclohexenone units are present, the R^1, R^2, and R^3 groups may vary independently.

[0034] Among the peptide analogs listed above, one preferred subclass is that defined by the formula



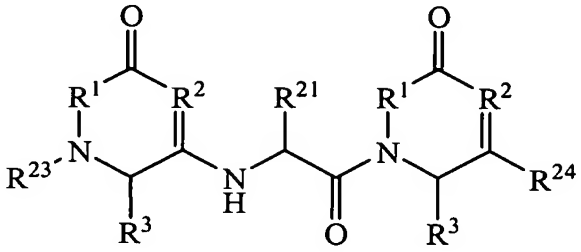
in which R^{24} is an activated leaving group, or OR^{25} where R^{25} is H or an oxygen-protecting group, or SR^{26} where R^{26} is H or an alkyl or aryl group. This subclass also includes amine-protected analogs of this formula.

[0035] Another preferred subclass is that defined by the formula



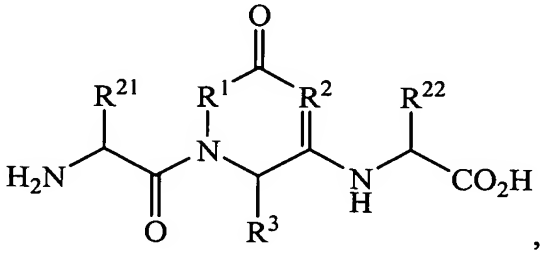
in which R^{23} is an amine protecting group, including carboxy-protected analogs of this formula.

[0036] A further preferred subclass is that defined by the formula



in which R^{23} is an amine protecting group and R^{24} is either an activated leaving group, OR^{25} where R^{25} is H or an oxygen-protecting group, or SR^{26} where R^{26} is H or an alkyl or aryl group.

[0037] A still further preferred subclass is that defined by the formula



,

including amine-protected analogs, carboxy-protected analogs, amine-protected and carboxy-protected analogs, and amine-protected and carboxy-activated analogs of this formula.

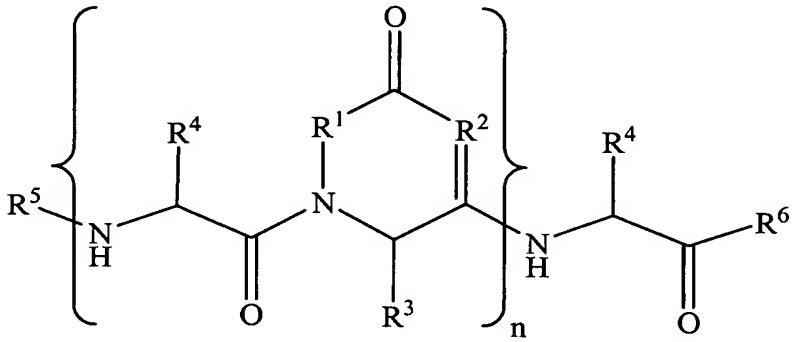
[0038] The peptide analogs within these preferred classes are useful as components in the synthesis of longer-chain peptide analogs. In certain embodiments of this invention, the R^{21} and R^{22} groups in these formulas are side chains, or protected side chains, of natural amino acids. In other embodiments, either R^{21}, R^{22}, or both are side chains, or protected side chains, of unnatural amino acids.

[0039] Further peptide analogs of this invention are defined as peptides in which at least one amino acid, but less than all amino acids, is replaced by an azacyclohexenone group described above. Preferred analogs are those containing from 2 to 200 amino acids and from 1 to 100 azacyclohexenone groups. More preferred are those that contain from 2 to 100 amino acids and from 1 to 50 azacyclohexenone groups, and most preferred are those that contain from 2 to 10 amino acids and from 1 to 20 azacyclohexenone groups. The number ratio of azacyclohexenone groups to amino acids in these analogs is preferably from 1:10 to 10:1, more preferably from 1:5 to 5:1, and most preferably from 1:2 to 1:1.

[0040] The remaining amino acids in the peptide analogs of this invention, i.e., those that have not been replaced by the azacyclohexenone units described above, can include any natural amino acids, amino acid analogs, amino acid mimetics, or any combination of these three groups. Preferably, the remaining amino acids are either all natural amino acids or a combination of natural and unnatural amino acids. Particularly preferred amino acids are those whose side chains are members of the preferred groups listed in the definition of "amino acid side chain" above.

[0041] In peptide analogs of this invention in which two or more amino acids are replaced by azacyclohexenone units, it is preferred that the azacyclohexenone units occupy non-adjacent positions along the peptide chain. In certain embodiments, every second amino acid is replaced by a corresponding α-substituted azacyclohexenone unit. An α-substituted azacyclohexenone unit is termed "corresponding" when the R^3 group is the same amino acid side chain as that of the amino acid that the α-substituted azacyclohexenone unit replaces.

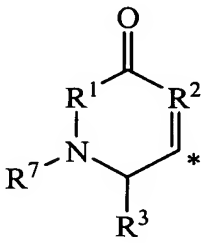
[0042] Still further peptide analogs of this invention are those defined by the following formula



in which:

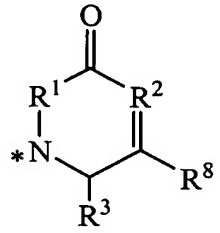
the R^4's are the same or different and each R^4 is H or an amino acid side chain;

R^5 is either a peptide chain terminating group or



in which R^7 is either H, alkyl, acyl, carbamoyl, or alkoxycarbonyl, and * denotes the site of attachment;

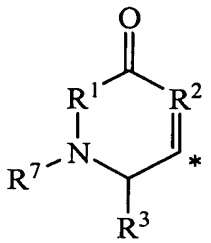
R^6 is either a peptide chain terminating group or



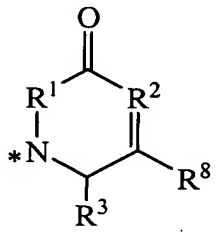
in which R^8 is either hydroxyl, alkoxy, mercapto, alkylmercapto, amino, alkylamino, dialkylamino, or arylamino, and * denotes the site of attachment; and

n is at least 2.

[0043] Preferred subclasses among these peptide analogs are those in which the R^4's are a combination of side chains of natural and unnatural amino acids, and optionally H as well, and those in which each R^4 is either H or a side chain of a natural amino acid. Further preferred subclasses are those in which R^5 is either H, alkyl, acyl, carbamoyl, or alkoxycarbonyl. A still further preferred subclass is that in which R^5 is



[0044] In terms of the R^6 group, a preferred subclass is that in which R^6 is either hydroxyl, alkoxyl, amino, alkylamino, dialkylamino, or arylamino, with hydroxyl and alkylamino (particularly C_1-C_4 alkylamino) most preferred. A still further preferred subclass is that in which R^6 is



[0045] Among the peptide analogs containing the symbol "n" as an index of chain length, a preferred subclass is that in which n is 2 to 100, a more preferred subclass is that in which n is 2 to 50, and a most preferred subclass is that in which n is 2 to 5.

[0046] Constructs or hybrids in accordance with this invention include two sequences linked together by a linkage that permits a β-turn, the first sequence being a sequence of amino acids joined together by amide bonds as in a conventional peptide, and the second sequence being a sequence of amino acids joined together by amide bonds as in the first sequence except that one or more, but not all, of the amino acids is replaced by an azacyclohexenone unit of the present invention. The azacyclohexenone unit(s), with the assistance of the covalent linkage, induces a β-sheet interaction between the two sequences and thereby induces and stabilizes the first sequence, i.e., the all-amino-acid sequence, in a β-strand conformation. In this configuration, the all-amino acid sequence is particularly effective in engaging in β-sheet interactions with other ("target") peptides and thus performing such functions as inhibiting the target peptides from entering into β-sheet interactions with further peptides and thereby inhibiting the biological activity of these target peptides, and various affinity-type functions such as extracting the target peptides from peptide mixtures or mixtures in general. The linkage can also assist the sequences to enter into a β-sheet interaction with a third peptide sequence and thereby inhibit the biological activity of the third sequence. In either case, the construct size (i.e., the lengths of the two segments) is not critical to the invention, but in preferred embodiments, the all-amino-acid segment will contain from 3 to 200 amino acids and in the segment containing both amino acids and azacyclohexenone units the total of the amino acids and azacyclohexenone units will range from 3 to 200. Ranges for both segments that are more preferred are 3 to 100, and most preferred are 3 to 20. The linkage between the segments can vary and is not critical to the invention except that, for segments that are linked head-to-tail by amino acid units, the linkage should not be one that is sterically or otherwise hindered from assuming a β-turn conformation. Preferred linkages are those that favorably assume or promote a β-turn conformation. Examples are D-proline-alanine (D-Pro-Ala) and asparagine-glycine (Asn-Gly).

[0047] In the constructs of this invention as well as the peptide analogs that contain α-substituted azacyclohexenone units and are intended to enter into β-sheet-like interactions with target peptides, the amino acids of the α-substituted azacyclohexenone-containing sequence are preferably those whose side chains are chosen on the basis of known side chain-

side chain affinities within β-sheets through design of sterically and electronically complementary structures, or by screening analogs. Likewise, the R^3 groups of the α-substituted azacyclohexenone units are preferably chosen on the basis of their affinities with the side chains of opposing amino acids in the β-sheet conformation. These affinities and their role in β-sheet-like interactions are known in the art. See, for example, Smith, C.K., et al., "Guidelines for Protein Design: The Energetics of β-Sheet Side Chain Interactions," *Science* **1995**, vol. *270*, 980; Ramirez-Alvarado, M., et al., "De novo design and structural analysis of a model β-hairpin peptide system," *Nature Structural Biology* **1996**, vol. *3*, 604; von Heijne, G., et al., "The β-Structure: Inter-Strand Correlations," *J. Mol. Biol.* **1997**, vol. *117*, 821. A further method for selecting the amino acids and the R^3 groups of the azacyclohexenone-containing sequence is to base the selection on a peptide sequence known to be complementary to the target peptide. Thus, in accordance with known principles, the side chains of the amino acids in the α-substituted azacyclohexenone-containing sequence preferably do not repel, but are instead compatible with, the side chains of the amino acids at the corresponding locations of the all-amino-acid segments or target peptides or proteins. This complementarity may result from a pairing of directly opposing residues but the affinity of any particular residue for an opposing residue may also be influenced by neighboring residues. Some of the ways in which directly opposing residues can be selected to achieve compatibility are the inclusion of basic side chains in the α-substituted azacyclohexenone-containing sequence to oppose acidic side chains in the conventional peptide (all-amino-acid) sequence, acidic side chains in the α-substituted azacyclohexenone-containing sequence to oppose basic side chains in the conventional peptide sequence, hydrophobic side chains in one sequence to oppose hydrophobic side chains in the other sequence, and hydrophilic side chains in the one sequence to oppose hydrophilic side chains in the other sequence. The characters of the side chains of known amino acids are well known to those skilled in the art and hence the appropriate selection for optimal favoring of β-sheet interaction will be readily apparent on this basis. The following is a rough characterization of several amino acids:

Side Chain Character	Amino Acids
acidic	aspartic acid, glutamic acid
basic	arginine, histidine, lysine
hydrophobic	alanine, isoleucine, leucine, methionine, phenylalanine, valine, tryptophan, tyrosine
hydrophilic	asparagine, glutamine, serine, threonine

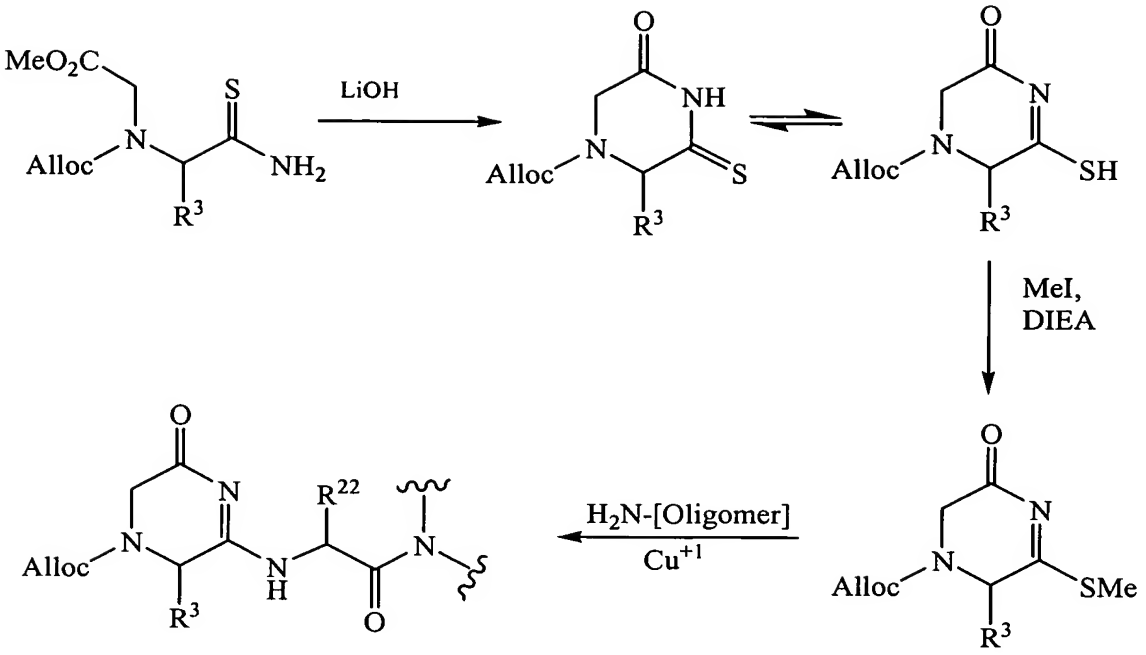
Synthesis of the Compounds, Peptide Analogs, and Constructs of the Invention

[0048] Abbreviations used in the following synthesis descriptions and examples are as follows:

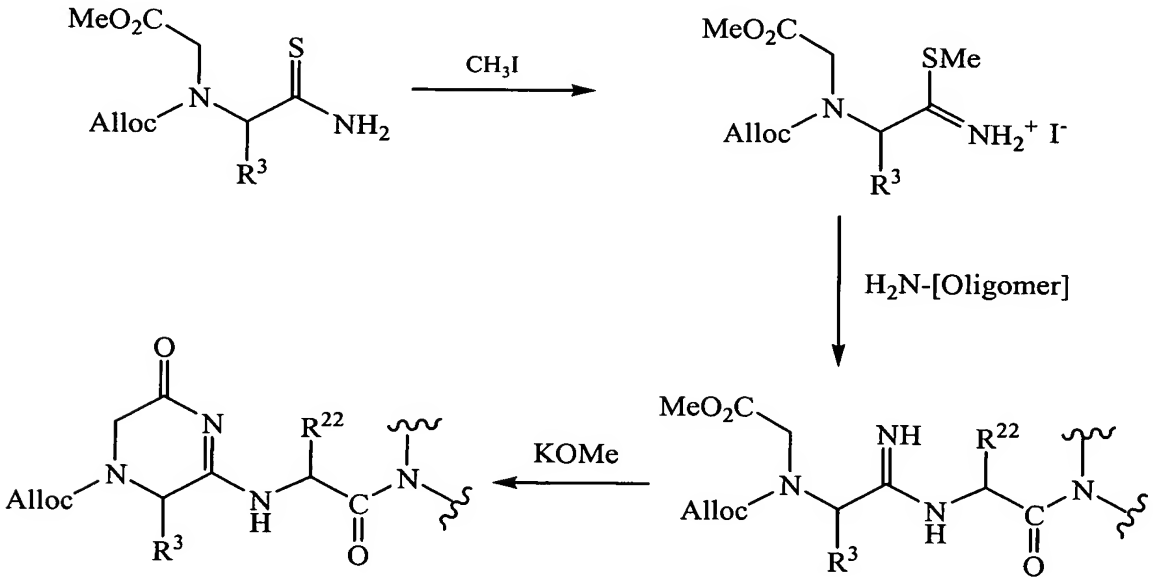
Ac	acetyl
Alloc	allyloxycarbonyl
Boc	*t*-butoxycarbonyl
Cbz	benzyloxycarbonyl
DCM	dichloromethane
DIEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EtOAc	ethyl acetate
EtOH	ethanol
HATU	*N*-[(dimethyl-amino)-1*H*-1,2,3-triazolo-[4,5-b]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluoro-phosphate *N*-oxide
iPr	isopropyl
LDA	lithium diisopropylamide

MeCN	acetonitrile
MeOH	methanol
Pf	9-phenyl-9-fluorenyl
Ph	phenyl
tBu	*t*-butyl
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography

[0049] The azacyclohexenones and their functionalized derivatives can be synthesized by conventional methods. The dihydropyrazinone (in which R^1 is CH_2 and R^2 is N), for example, can be prepared from an amide of an amino acid whose side chain is the same as, or a protected version of, R^3 sought for the product. The amide is thionated preferentially relative to the carbamate and ester groups with Lawesson's reagent using known methods as reported by Cava, M.P., et al., *Tetrahedron* **1985**, vol. 41, 5061-5087. The thioamide can then be cyclized by base-induced cyclization while protecting the stereocenter, as reported by Koehler, W., et al., *Chem. Ber.* **1967**, vol. 100, 1073-1081. The cyclic thioamide is then activated by S-methylation, followed by reaction with an amino acid or peptide derivative to give the dihydropyrazinone peptide analog. This reaction scheme is shown below:

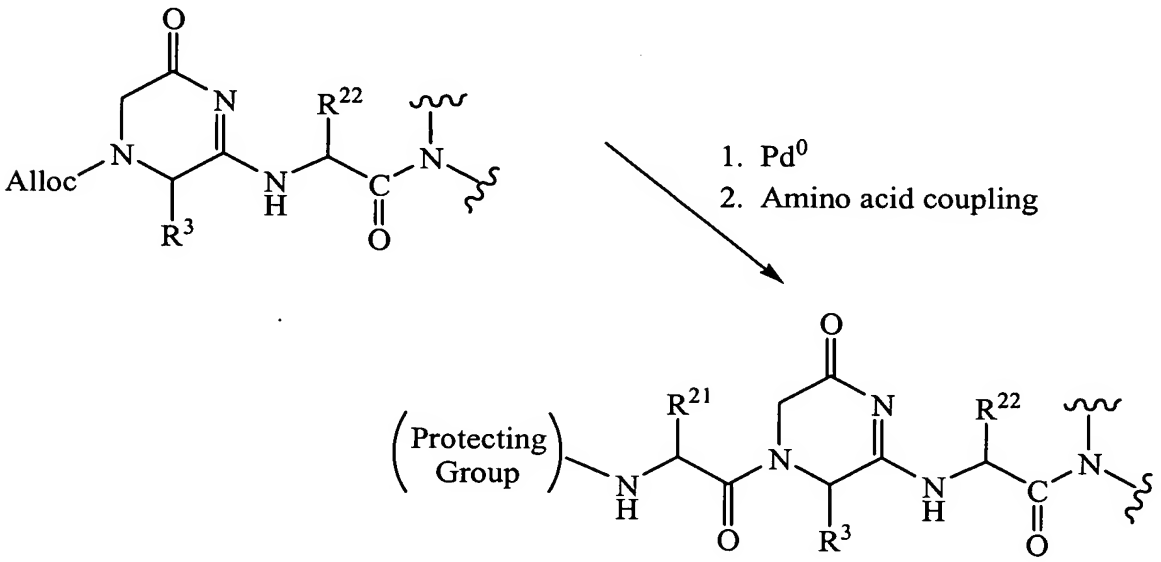


[0050] In an alternative route, the amide is first converted to a thioimidate salt which is then converted to the amidine under neutral conditions. The amidine can then be cyclized at the less hindered nitrogen to give the dihydropyrazinone peptide analog.



[0051] In either scheme, the initial methyl ester can be replaced by a more reactive ester leaving group to further facilitate the cyclization. Additionally in either scheme, the allyloxycarbonyl protecting group can be replaced with other nitrogen-protecting groups, such as benzyloxycarbonyl (Cbz) or fluorenylmethoxycarbonyl (Fmoc).

[0052] The dihydropyrazinone peptide analog can be chain-elongated by removal of the nitrogen protecting group and coupling with a nitrogen-protected amino acid derivative under the conventional conditions for this type of reaction, as will be readily apparent to those skilled in the art. This sequence of reactions is shown below:



[0053] The α-substituted dihydropyridinone (in which R^1 is CH_2 and R^2 is CH) can be prepared from an amino alcohol precursor, examples of which are commercially available or readily obtained from natural and unnatural amino acids by reduction of the carboxyl group. Alkylation of the amino group with methyl bromoacetate is followed by cyclization to the lactone. The lactone is reacted with 9-phenylfluorenyl bromide to introduce the 9-phenyl-9-fluorenyl ("Pf") protecting group on the nitrogen; the Pf group is known in the art for its ability to inhibit racemization at the adjacent carbon stereocenter (Lubell, W.D., Rapoport, H., *J. Am. Chem. Soc.* **1987**, vol. *109,* 236). Other protecting groups commonly employed in peptide synthesis may also be used. Reaction of the protected lactone with the lithium salt of diethyl methylphosphonate affords the ketophosphonate, the alcohol of which is oxidized to the aldehyde with the Swern reagent (Mancuso, A.J.; Swern, D. *Synthesis*, **1981**, 165-185). The aldehyde is cyclized on treatment with base, as described by Grison, C., et al., *Tetrahedron* **2001**, vol. *57*, 4903; Hintermann, T., et al., *Helv. Chim. Acta* **1998**, vol. *81*, 983. Conversion of the enone functionality to the β–phenylthioenone is accomplished using the procedure of De Groot, A., et al., *Synth. Commun.* **1987**, vol. *17*, 1607. The activated dihydropyridinone is coupled with an amino acid ester with catalysis by Cu^{+1} or a lanthanide Lewis acid such as Yb^{+3}. The coupled product may be further extended at either the N-terminus or C-terminus

by selective deprotection and conventional coupling reactions with amino acid derivatives or with azacyclohexenone derivatives. An illustrative reaction scheme is shown in FIG. **1**.

[0054] The dihydropyridazinone (in which R^1 is NH and R^2 is CH) can be prepared from a natural or unnatural amino acid. The nitrogen-protected form is aminated by the method of Vidal, J., et al., *Tetrahedron Lett.* **1998,** vol. *39*, 8845, and the carbonyl group is converted to the β-ketoester using the method of Brooks, D.E., et al., *Angew. Chem. Intl. Ed. Engl.* **1979**, vol. *18*, 72. Cyclization is induced by base and the carbamate protecting group is either cleaved under these conditions or removed in a separate step. The dihydropyridazinone may be coupled directly with an amino acid by heating in an alcoholic solvent, or it may be activated by reaction with, for example, mesitylenesulfonyl chloride, followed by coupling with an amino acid ester with catalysis by a lanthanide Lewis acid such as Yb^{+3}. The coupled product may be further extended at either the N-terminus or C-terminus by selective deprotection and conventional coupling reactions with amino acid derivatives or with azacyclohexenone derivatives. An illustrative reaction scheme is shown in FIG. **2.**

[0055] The dihydrotriazinone (in which R^1 is NH and R^2 is N) can be prepared from a natural or unnatural amino acid. The amino acid is first aminated as illustrated in the synthesis of the dihydropyridazinone. The carboxyl group is then converted to the thionoamide, which is cyclized with base. The thioimide is alkylated with methyl iodide and then coupled to an amino acid derivative or a peptide derivative with catalysis by Cu(I) or a soft Lewis. Alternatively, the thioamide may be alkylated with methyl iodide and the thioamidate salt can be converted to the amidine under neutral conditions. The amidine can then be cyclized at the less hindered nitrogen to give the triazinone. An illustrative reaction scheme is shown in FIG. **3**.

[0056] Each of the products of the reactions shown in FIGS. **1**, **2**, and **3** can be N-deprotected and coupled to another amino acid using conventional procedures. Alternatively, the azacyclohexenone-amino acid ester derivatives depicted in FIGS. **1** and **2** can be deprotected at the carboxyl group and the resulting acid then coupled as a unit for more rapid chain elongation.

[0057] The α-substituted azacyclohexenone enantiomers that correspond to the L configuration of the natural amino acids can be obtained through synthesis, beginning with an amino acid or amino acid derivative of the natural configuration as starting material, as outlined in the Examples below. Alternatively, if synthetic intermediates are produced in

racemic form, they may be resolved into the separate enantiomers prior to incorporation in the peptide analog. The desired stereoisomer may also be obtained from peptide analogs in which both enantiomers of an α-substituted azacyclohexenone are present as a mixture, using chromatography or other means known in the art.

[0058] Coupling can also be performed by solid phase synthesis. For example, an Fmoc-protected amino acid coupled to a solid resin such as a Wang or Merrifield polystyrene resin can be deprotected with 20% piperidine in DMF, then coupled to an activated and N-protected form of the azacyclohexenone in a mixed solvent of methylene chloride and DMF (1:3.5 volume ratio), followed by treatment with acetic anhydride, DIEA, and methylene chloride (1:1:3). The N-protecting group is then removed, and the steps repeated until the desired peptide analog chain is achieved.

[0059] Constructs consisting of an all-amino-acid segment linked to a segment in which one or more (but not all) amino acids are replaced by an azacyclohexenone group are readily synthesized by methods analogous to those described above, with the β-turn-promoting linkage added at the appropriate site. Solid phase synthesis is readily used, and the azacyclohexenone units can be incorporated at either the N-terminus or the C-terminus of the hybrid. C-Terminal azacyclohexenone incorporation, for example, can be performed by solid-phase synthesis of the desired azacyclohexenone-containing segment, followed by coupling to amino acids using standard peptide coupling reactions. N-terminal azacyclohexenone incorporation can be performed by first synthesizing the solid phase-bound peptide segment, followed by coupling to the azacyclohexenone-containing segment.

Formulas and Administration of the Peptide Analogs and Constructs of the Invention

[0060] When used as drugs for administration to mammals, the compounds of this invention can be administered in water-soluble form and preferably as pharmaceutically acceptable salts. Pharmaceutically acceptable salts are those that retain the biological effectiveness of the free bases or acids without introducing unfavorable side effects. The salts can be either acid or base addition salts, depending on the peptide analog itself. Examples of acceptable acid addition salts are those formed with inorganic acids such as hydrochloric, hydrobromic, sulfuric, nitric, or phosphoric acid, and those formed with organic acids such as acetic, propionic, glycolic, pyruvic, oxalic, maleic, malonic, succinic, fumaric, tartaric, citric, benzoic, cinnamic, mandelic, methanesulfonic, ethanesulfonic,

p-toluenesulfonic, or salicylic acid. Examples of acceptable base addition salts are those formed with inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, manganese, or aluminum hydroxide, and those formed with organic bases such as primary, secondary, and tertiary amines such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine, or with basic ion exchange resins.

[0061] The compounds can be formulated into suitable pharmaceutical preparations for administration by intravenous injection, intramuscular injection, intravenous infusion, oral administration, or any other conventional methods of administration. The active ingredient can be compounded, for example, with non-toxic, pharmaceutically acceptable carriers and excipients that are common to pharmaceutical formulations in general, as aqueous solutions, or as emulsions or suspensions, or in solid or semi-solid forms such as tablets, pellets, capsules, or suppositories. Examples of suitable carriers are water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea. Excipients can include agents for stabilization, thickening, coloring, or scent, or agents to aid in formulating the dosage forms, selected as needed in accordance with the intended manner of administration as well as the particular condition to be treated. Tablets for oral administration, for example, can contain microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate, or glycine, along with any of various disintegrants such as corn, potato, or tapioca starch, alginic acid or complex silicates, together with granulation binders such as polyvinylpyrrolidone, sucrose, gelatin or acacia. Lubricating agents such as magnesium stearate, sodium lauryl sulfate or talc can also be included.

[0062] The amount of active ingredient to be included in a single dosage form will vary depending on the patient to be treated and the particular mode of administration. The optimal dose level for a particular patient will depend on such factors as the age, body weight, general health, sex, and diet of the patient, as well as the time of administration, the route of administration, the rate of excretion, the severity of the disease being treated, and whether or not the patient is simultaneously undergoing any other drug therapy. In most cases, the amount of active ingredient administered will range from about 1 to about 1,000 mg per day, preferably from about 10 to about 500 mg per day.

[0063] The following examples are offered for purposes of illustration, and are not intended to impose limits on the scope of the invention.

[0064] Reagents and solvents were obtained from commercial suppliers and were used as received unless otherwise noted. Dichloromethane, MeCN, TEA, and DIEA were distilled from calcium hydride, THF was distilled from Na. Solvents were removed with a rotary evaporator at aspirator pressure, followed by drying under high vacuum. Flash chromatography was performed according to the method described by Still et al. (Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923) using 60 Å pore, 200-mesh silica gel from E. Merck & Co. and the solvent system indicated. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F_{254} plates.

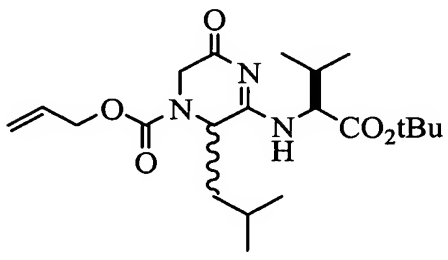
[0065] Analytical high pressure liquid chromatography (HPLC) analysis was performed using a Waters 996 Photodiode Array Detector with a Waters 600 Controller and Pump. The analytical column was a Varian DYNAMAX-100 Å (4.6 mm × 250 mm) reversed-phase C_{18} column. Retention times were recorded using the gradient method A (flow rate 1 mL/min). Chiral analytical HPLC was performed using an Hewlett Packard Series 1100 Quatpump and MWD. The analytical column used was a Chiralcel OD normal phase column. Retention times were recorded using a solvent system of hexanes/isopropanol 85:15. Preparative HPLC purification was performed using a Waters 486 Tunable Absorbance Detector and a Waters 600 Controller and Pump. The column was a Varian DYNAMAX-100 Å (21.4 mm × 250 mm) reversed-phase C_{18} column equipped with a guard column. For purification purposes, the gradient program used was Method B (flow rate 15 mL/min). Liquid Chromatography-Mass Spectometry (LCMS) analysis was performed using an HP Series 1100 Quatpump, MWD, and MSD. The analytical column used was a Zorbax SB-C18 reverse-phase column (2.1 mm × 5 cm). The gradient method C was used for analysis (flow rate 0.4 mL/min).

HPLC Method A		HPLC Method B		LCMS Method	
Time	% CH_3CN (+0.1% TFA)	Time	% CH_3CN	Time	% CH_3CN (+0.1% TFA)
0	10	0	0	0	5
0-25	Ramp to 95	0-40	Ramp to 30	0-8	Ramp to 95
30	95	40-45	Ramp to 95	8-10	95
32	Ramp to 10	50	95	10-12	Ramp to 5
		55	Ramp to 0	12-14	5

[0066] Infrared (IR) spectra were recorded with a Perkin-Elmer 1600 Fourier transform infrared spectrometer and are reported in wavenumbers (cm^{-1}). Nuclear Magnetic Resonance (NMR) spectra were obtained using Bruker 300, 400, or 500 MHz spectrometers with ^{13}C operating frequencies of 100 and 125 MHz. Spectral data are reported as chemical shifts (multiplicity, number of hydrogens, coupling constants in Hz). Chemical shifts were calibrated to the solvent peak (3.31 for CD_3OD, 7.27 ppm for $CDCl_3$, 2.50 for $(CD_3)_2SO$). Peaks for the major rotamer are reported, and representative peaks of the minor rotamer are also reported. All ^{13}C NMR were proton decoupled and referenced to $CDCl_3$ (77.0 ppm) or $(CD_3)_2SO$ (39.5 ppm).

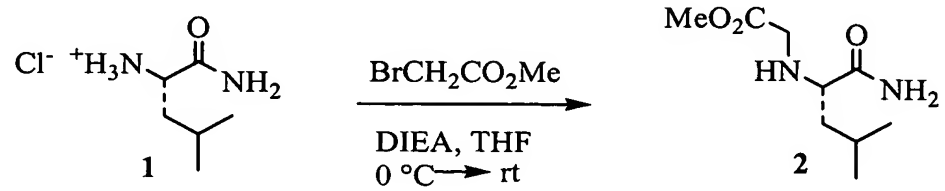
EXAMPLE 1

[0067] This example illustrates the liquid-phase synthesis of a two-unit construct of the present invention that includes an N-protected dihydropyrazinone of the present invention linked to a carboxy-protected valine residue. The construct is (6*RS*)-6-isobutyl-5-((1*S*)-1-*t*-butoxycarbonyl-2-methylpropylamino)-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic acid allyl ester, or by an abbreviated name Alloc-[Leu]-Val t-butyl ester in which the brackets denote the dihydropyrazinone counterpart of the amino acid (in this case, leucine), and its formula is as follows:



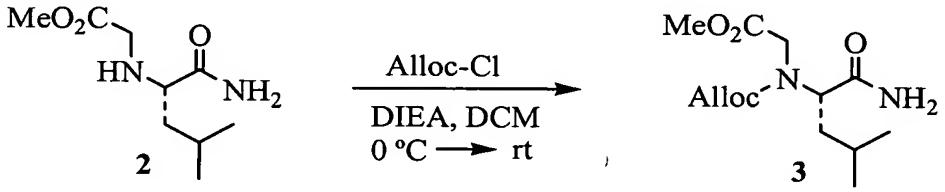
[0068] Sections A through F below illustrate one reaction scheme to this construct.

1.A. N-(2-Methoxy-2-oxoethyl)-L-Leucine Amide (2) from L-Leucine Amide Hydrochloride (1)



[0069] To 2.0 g (12 mmol) of L-leucine amide hydrochloride (**1**) in 20 mL of dry THF at 0 °C was added 5.0 mL (29 mmol) of DIEA followed by slow addition of a solution of methyl bromoacetate (1.4 mL, 14 mmol) in dry THF (20 mL). The reaction solution was stirred under argon and allowed to warm up to room temperature over a 20 h period before it was diluted with 50 mL of saturated NH_4Cl. After concentration of the reaction mixture, the product was extracted with EtOAc (2 × 50 mL). The organic layer was further washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (DCM/MeOH/TEA 99:1:1→95:5:1) to afford **2** as a yellow oil (1.1 g, 47% yield). If the column chromatography is performed without TEA, the product is isolated as a white solid. R_f = 0.26 ($KMnO_4$/basic, DCM/MeOH 95:5); retention time (analytical HPLC) = 13.1 min; IR (film) 1673, 1745, 2955 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, 3, J = 4.7), 0.92 (d, 3, J = 4.7), 1.44 (m, 1, J = 5.5, 8.8, 14), 1.54 (m, 1, J = 4.8, 8.8, 14), 1.73 (m, 1), 2.1 (s, 1), 3.08 (dd, 1, J = 4.8, 8.8), 3.29 (d, 1, J = 17.5), 3.43 (d, 1, J = 17.5), 3.68 (s, 3), 6.42 (s, 1), 7.00 (s, 1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.8, 23.2, 24.9, 43.1, 49.2, 51.9, 61.2, 172.6, 178.1; MS (ES) m/z 203.1 ($M+H^+$), 158.1 ($M+H^+$-$CONH_2$); HRMS (FAB) m/z 203.1398 ($M+H^+$ $C_9H_{20}N_2O_3$ requires 203.1396).

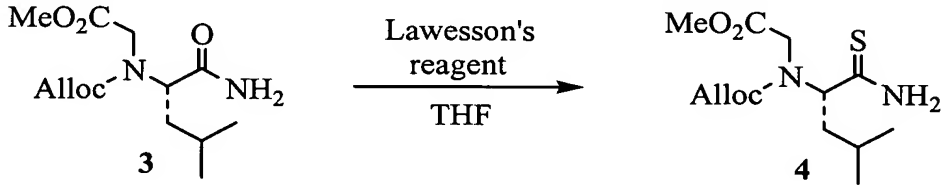
1.B. N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Leucine Amide (3) From N-(2-Methoxy-2-oxoethyl)-L-Leucine Amide (2)



[0070] To 0.37 g (1.8 mmol) of **2** in 18 mL of dry DCM at 0 °C was added 2.0 mL (11.1 mmol) of DIEA followed by slow addition of allyl chloroformate (0.55 mL, 5.2 mmol). The reaction solution was stirred under argon and allowed to warm up to room temperature over a

20 h period before it was diluted with 50 mL of saturated NH_4Cl. After concentration of the reaction mixture, the product was extracted with EtOAc (3 × 10 mL). The organic layer was further washed with saturated NH_4Cl (2 × 15 mL), saturated $NaHCO_3$ (15 mL), and brine (15 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 1:2 → 1:1 → 2:1) to afford **3** as a yellow oil (0.41 g, 79% yield). R_f = 0.51 (ninhydrin, I_2, EtOAc/hexanes 10:3); ^1H NMR (500 MHz, $CDCl_3$, rotamers) δ 0.92-0.99 (m, 6), 1.50-1.59 (m, 1), 1.59-1.66 (m, 1, J = 4.0), 1.79-1.85 (m, 0.6, J = 5.5), 1.90-1.96 (m, 0.5, J = 4.5), 3.74 (s, 1.6), 3.79 (s, 1.4), 3.95-4.06 (m, 2), 4.42 (m, 0.4), 4.59 (dt, 1, J = 1.5, 5.5), 4.63 (dd, 1, J = 4.2, 5.8), 4.71 (dd, 0.7, J = 6.0, 9.0), 5.19-5.35 (m, 2, J = 1.5) 5.84-5.90 (m, 1), 6.44 (s, 0.6), 6.54 (s, 0.4), 7.28 (s, 0.6), 7.78 (s, 0.4); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ 21.3/21.7, 23.0/23.2, 24.7/24.7, 37.5/38.2, 45.9/#, 52.4/52.7, 57.7/59.3, 66.7/67.0, 117.6/118.4, 131.8/132.1, 155.8/155.8, 171.0/171.8, 173.2/173.6; MS (ES) m/z 309.2 ($M+Na^+$), 242.1 ($M+H^+$-$CONH_2$); HRMS (FAB) m/z 287.1611 ($M+H^+$ $C_{13}H_{23}N_2O_5$ requires 287.1607).

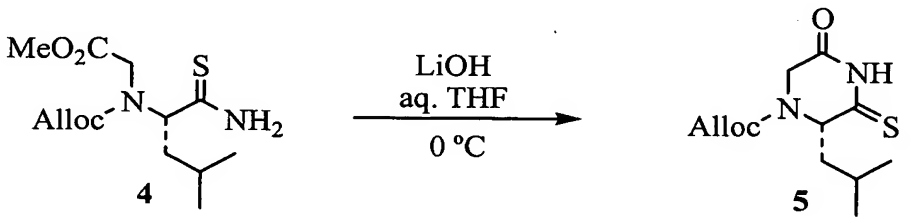
1.C. N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Leucine Thioamide (4) From N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Leucine Amide (3)



[0071] To 1.0 g (3.5 mmol) of N-allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-leucine amide **3** in 50 mL of dry THF was added 0.99 g (2.4 mmol) of Lawesson's reagent, which turns the solution faint yellow and opaque. The reaction solution was stirred under argon at room temperature, and the reaction was stopped at 1.5 h when no more starting material was observed by TLC. After concentration of the reaction mixture, the crude product was redissolved in EtOAc. The organic layer was washed with saturated $NaHCO_3$ (2 × 50 mL). The basic aqueous layer was acidified by addition of solid $KHSO_4$ and extracted with EtOAc (2 × 20 mL). The combined organic layers were further washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 1:2 → 1:1) to afford **4** as a white solid (0.82 g, 78% yield). R_f = 0.55 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 16.2 min; retention time (chiral HPLC): 7.1 min; ^1H NMR (500 MHz, $CDCl_3$, rotamers) δ 0.94-0.96 (m, 6), 1.46-1.56 (m, 1), 1.64-1.72 (m, 1), 2.22 (m, 0.5, J = 6.0, 8.6, 14.5), 2.47 (m, 0.5, J = 4.0, 10.7,

14.8), 3.75 (s, 1.5), 3.82 (s, 1.5), 3.83 (m, 1), 4.09 (t, 1, J = 18.0), 4.61 (dd, 1, J = 1.4, 5.4), 4.65 (t, 1, J = 5.4), 4.86 (dd, 0.4, J = 3.6, 11.1), 4.93 (dd, 0.6, J = 6.1, 9.2), 5.21-5.35 (m, 2, J = 1.3), 5.82-5.92 (m, 1), 7.60 (s, 0.5), 7.68 (s, 0.5), 8.66 (s, 0.5), 9.53 (s, 0.5); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ 20.9/21.8, 22.9/23.4, 25.0/25.2, 40.3/41.4, 45.6/46.3, 52.5/53.0, 62.8/64.9, 67.0/67.3, 117.8/118.8, 131.6/132.0, 156.0/156.4, 171.1/172.5, 207.0/207.7; MS (ES) m/z 303.1 ($M+H^+$); HRMS (FAB) m/z 303.1373 ($M+H^+$ $C_{13}H_{23}N_2O_4S$ requires 303.1378).

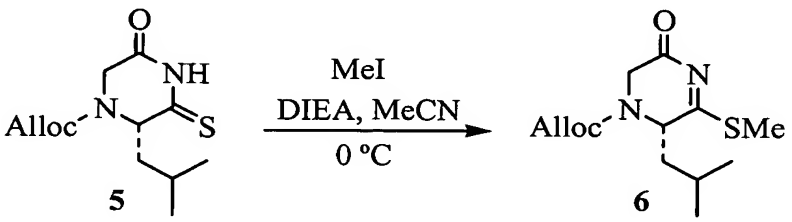
1.D. (2*S*)-2-Isobutyl-5-oxo-3-thioxopiperazine-1-carboxylic Acid Allyl Ester (5) From N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Leucine Thioamide (4)



4 5

[0072] To 0.24 g (0.78 mmol) of **4** in 16 mL of THF/H_2O 3:1 at 0 °C was added 1 M LiOH (0.78 mL, 0.78 mmol) dropwise. The reaction solution was stirred under nitrogen and quenched after 6 min by addition of solid $KHSO_4$. After concentration of the reaction mixture, the aqueous solution was extracted with EtOAc (2 × 10 mL). The organic layer was further washed with 1 M $KHSO_4$ (3 × 10 mL), saturated $NaHCO_3$ (3 × 10 mL), and brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. No further purification was necessary to afford **5** as a yellow oil (0.15 g, 71% yield). R_f = 0.6 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 15.8 min; retention time (chiral HPLC): 8.0 min; ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 (d, 3, J = 6.5), 0.94 (d, 3, J = 5.9), 1.58-1.74 (m, 3), 3.6-3.8 (m, 1), 4.58 (m, 2, J = 4.8), 4.8-5.0 (m, 1), 5.18 (dd, 1, J = 1.1, 10.4), 5.26 (dd, 1, J = 1.3, 17.2), 5.2-5.4 (m, 1), 5.8 (m, 1, J = 5.6, 10.8), 10.4 (s, 1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.0, 22.8, 24.6, 41.2, 42.2, 61.1, 67.0, 118.5, 131.6, 154.0, 165.1, 206.5; MS (ES) m/z 271.1 ($M+H^+$).

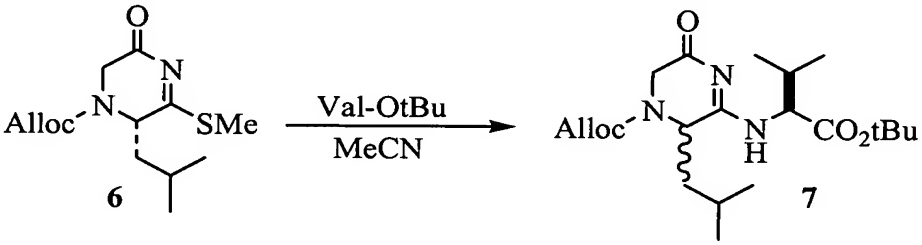
1.E. (6*S*)-6-Isobutyl-5-methylsulfanyl-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic Acid Allyl Ester (6) From (2*S*)-2-Isobutyl-5-oxo-3-thioxopiperazine-1-carboxylic Acid Allyl Ester (5)



5 6

[0073] To 0.11 g (0.4 mmol) of **5** in 15 mL of dry MeCN at 0 °C was added 25 μL (0.4 mmol) of methyl iodide followed by slow addition of a solution of DIEA (80 μL, 0.46 mmol) in 10 mL MeCN via an addition funnel over the course of 30 min while the reaction solution was stirred under argon. After all of the base was added, the flask was capped and the solution was stirred for an additional 2 h. After concentration of the reaction mixture, the crude product was redissolved in EtOAc. The organic layer was washed with NaH_2PO_4 (2 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was used without further purification. R_f = 0.52 (UV, EtOAc/hexanes 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (m, 6, J = 4.4), 1.4-1.5 (m, 1), 1.6-1.7 (m, 2), 2.51 (s, 3), 3.9-4.0 (m, 1), 4.58-4.63 (m, 1), 4.62 (d, 2, J = 4.8), 4.73-4.74 (m, 0.5), 4.85-4.95 (m, 0.5), 5.26 (d, 1, J = 10.4), 5.32 (d, 1, J = 17.2), 5.92 (m, 1, J = 5.6, 10.8); MS (ES) m/z 285.1 ($M+H^+$).

1.F. (6*S*)-6-Isobutyl-5-((1*S*)-1-*t*-butoxycarbonyl-2-methylpropylamino)-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic acid Allyl Ester (7) From (6*S*)-6-Isobutyl-5-methylsulfanyl-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic Acid Allyl Ester (6)

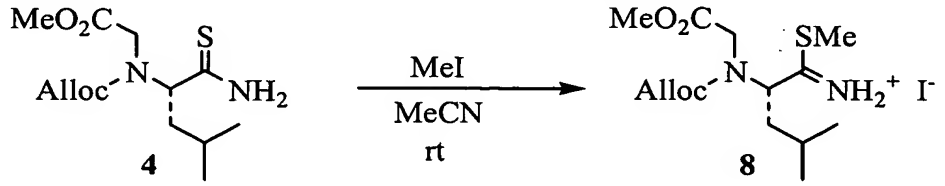


[0074] To a solution of crude acyl thioimidate **6** (97.4 mg, 0.34 mmol) in 5 mL of dry MeCN was added 1.1 equivalents of valine t-butyl ester, and the reaction solution was allowed to stir under argon overnight. After concentration of the reaction mixture, the crude product was purified by flash chromatography (DCM/MeOH 99:1) to afford **7** as a yellow oil (36% yield over two steps, 5/1 ratio of *S*/*R* stereoisomers of the dihydropyrazinone unit).

EXAMPLE 2

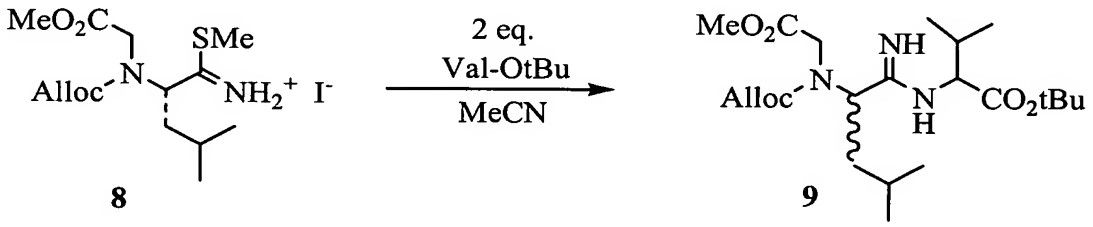
[0075] This example illustrates an alternative synthetic route to the construct prepared in Example 1. This route begins with Sections 1.A through 1.C of Example 1 and continues with Sections 2.D through 2.F below.

2.D. N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Leucine Methylthioimidate (8) From N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Leucine Thioamide (4)



[0076] To 0.05 g (0.16 mmol) of **4** in 5 mL of dry MeCN was added 31 μL of (0.50 mmol) methyl iodide and the reaction solution was stirred under argon at room temperature for 4 h. After concentration of the reaction mixture, the crude product (**8**) was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$, crude, rotamers/diastereomers) δ 0.86-0.93 (m, 6), 1.48-1.56 (m, 1), 1.73 (m, 1, J = 4.6, 9.4, 14.0), 2.05 (m, 0.3, J = 3.6, 10.6, 14.3), 2.13 (m, 0.7, J = 3.9, 10.4, 14.4); 2.92 (s, 2), 2.94 (s, 1), 3.74 (s, 3), 4.15-4.29 (m, 2), 4.50 (d, 1.3, J = 5.5), 4.56 (d, 0.7, J = 6.5), 5.14-5.23 (m, 3), 5.75 (m, 0.7, J = 5.4, 10.7, 16.1), 5.8-5.85 (m, 0.3); MS (ES) m/z 317.3 ($M+H^+$).

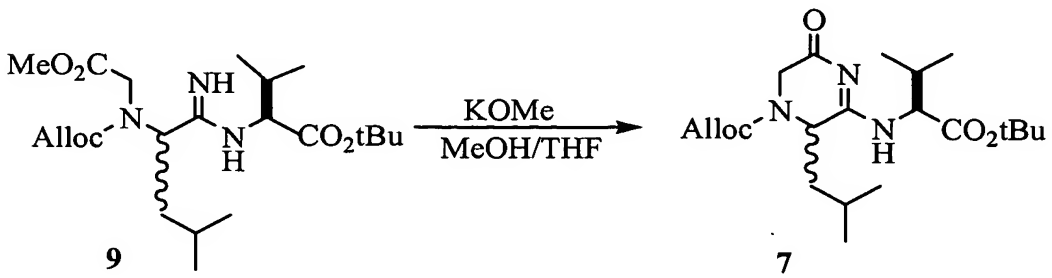
2.E. 2-(N-(Allyloxycarbonyl)-N-(2-methoxy-2-oxoethyl)amino)-4-methylpentanimidoyl)-L-Valine *t*-Butyl Ester (9) From N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Leucine Methylthioimidate (8)



[0077] To a crude product mixture containing 44.8 mg (01.4 mmol) of **8** in 1 mL of dry DCM was added 1.1 equivalents of valine t-butyl ester, and the reaction solution was stirred under argon for 22 h. After concentration of the reaction mixture, the crude product was purified by flash chromatography (EtOAc/hexanes 1:1 → EtOAc → MeOH/EtOAc 1:9) to afford **9** as a yellow oil (46.7 mg, 75% yield over two steps). R_f = 0.13 (UV, EtOAc/hexanes 1:1); ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.85-1.0 (m, 12), 1.44 (s, 9), 1.50-1.60 (m, 1), 1.55-1.75 (m, 1), 1.85-1.95 (m, 1), 2.20 (m, 1), 3.70 (s, 3), 4.15-4.40 (m, 3), 4.55 (s, 2), 4.75-4.90 (m, 1), 5.17-5.25 (m, 2), 5.80-5.95 (m, 1), 9.17 (s, 1), 9.48 (s, 1); MS (ES) m/z 442.4 ($M+H^+$).

2.F. (6*S*)-6-Isobutyl-5-((1*S*)-1-*t*-butoxycarbonyl-2-methylpropylamino)-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic acid Allyl Ester (7) From 2-(N-

(Allyloxycarbonyl)-N-(2-methoxy-2-oxoethyl)amino)-4-methylpentanimidoyl)-L-Valine *t*-Butyl Ester (9)



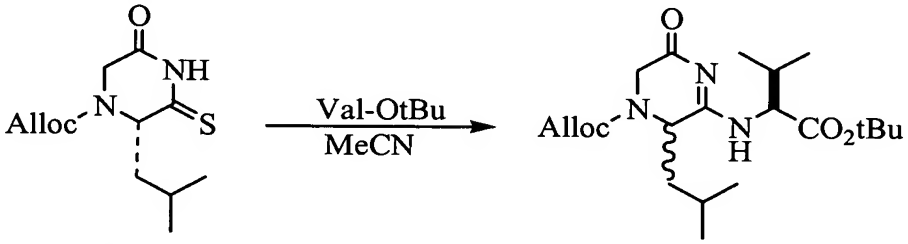
9 7

[0078] To a solution of 1.09 g (2.5 mmol) of the amidine **9** in 150 mL of 5:1 THF/MeOH at 0°C was added 0.2 g (2.8 mmol) of potassium methoxide. The reaction solution was allowed to stir under nitrogen and then neutralized after 20 min by addition of 1 M $KHSO_4$. The organic layer was washed with $KHSO_4$ (50 mL). The acidic aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with NaCl (1 × 50 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product **7** was used without further purification (0.71 g, 70% yield, 2/1 ratio of *S*/*R* stereoisomers of the dihydropyrazinone unit).

EXAMPLE 3

[0079] This example illustrates an alternative synthetic route to the construct prepared in Example 1. This route begins with Sections 1.A through 1.D of Example 1 and continues with Section 3.E below.

3.E. (6*S*)-6-Isobutyl-5-((1*S*)-1-*t*-butoxycarbonyl-2-methylpropylamino)-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic acid Allyl Ester (7) From (2*S*)-2-Isobutyl-5-oxo-3-thioxopiperazine-1-carboxylic Acid Allyl Ester (5)

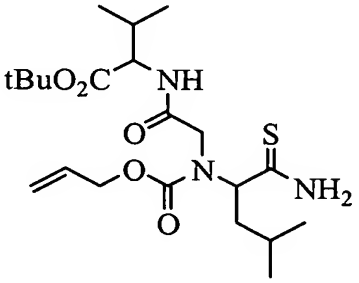


5 7

[0080] To a solution of thioimide **5** (50 mg, 0.18 mmol) in 5 mL of isopropanol at 50 °C was added valine t-butyl ester (1 equivalent), and the reaction solution was allowed to stir under nitrogen overnight. After concentration of the reaction mixture, the crude product was purified by flash chromatography (EtOAc/hexanes 1:3 → 1:2 → 1:1) to afford racemic starting material **5** (27% yield) and **7** as a yellow oil (17%yield, 1/2 ratio of *S*/*R* stereoisomers

of the dihydropyrazinone). R_f = 0.39 (UV, EtOAc/hexanes 1:1); IR (film) 1390.6, 1709.8, 2872.8, 2961.1 cm^{-1}; ^1H NMR (500 MHz, $(CD_3)_2SO$, 30°C, rotamers and diastereomers) δ 0.89-0.97 (m, 12), 1.26-1.30 (m, 1, J = 9.7), 1.4 (s, 9), 1.50-1.60 (m, 1), 1.65-1.75 (m, 1, J = 10.8), 2.13 (m, 1, J = 6.5), 3.68 (d, 0.4, J = 18.3), 3.78 (d, 0.6, J = 18.0), 4.19 (d, 1, J = 18.4), 4.33 (t, 0.25, J = 8.1), 4.40 (t, 0.75, J = 6.7), 4.57 (s, 2), 5.00-5.10 (m, 1), 5.19-5.28 (m, 2), 5.85-6.00 (m, 1), 8.80 (d, 0.25, J = 7.5), 8.98 (d, 0.3, J = 7.9), 9.02 (d, 0.45 J = 6.8); MS (ES) m/z 410.2 ($M+H^+$), 354.3 (M+H+-tBu).

[0081] A side product of the reaction of Section 3.E is N-(N-allyloxycarbonyl-N-(3-methyl-1-thiocarbamoylbutyl)-glycyl)-L-valine *t*-butyl ester, whose structure and confirmatory data are shown below:



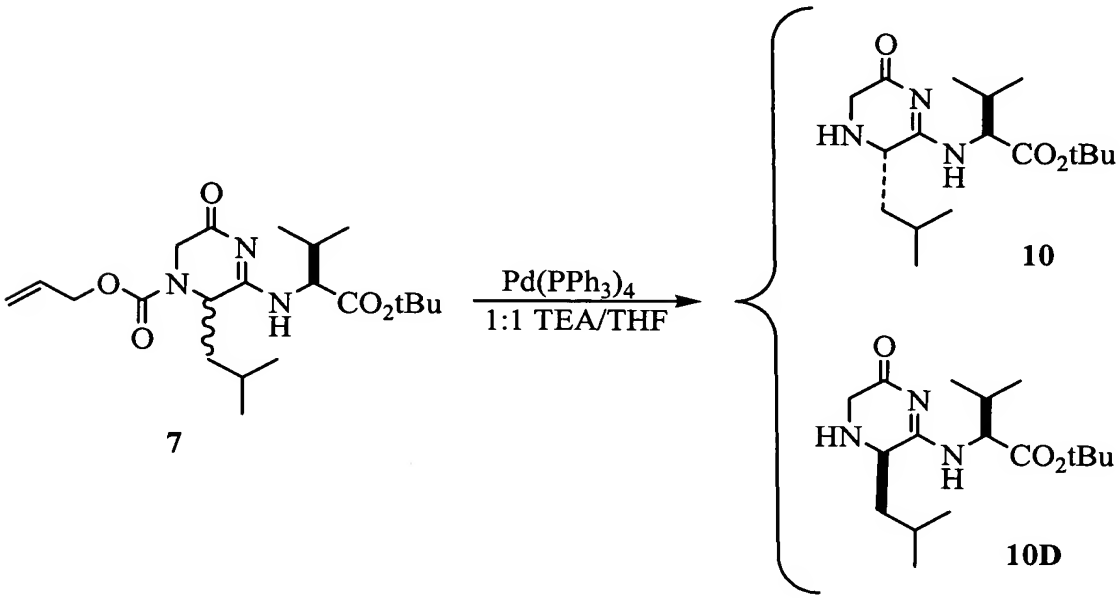
R_f = 0.55 (UV, EtOAc/hexanes 1:1); ^1H NMR (300 MHz, $(CD_3)_2SO$, rotamers/diastereomers) δ 0.87-0.93 (m, 12), 1.42 (s, 9), 1.60-1.80 (m, 2), 1.90-2.10 (m, 2), 4.00-4.15 (m, 3), 4.46-4.50 (m, 2), 4.50-4.70 (m, 1), 5.08-5.24 (m, 2), 5.75-5.95 (m, 1), 8.43 (d, 1, J = 9.0), 9.72 (s, 0.6), 9.75 (s, 0.4), 10.34 (s, 0.6), 10.52 (s, 0.4); MS (ES) m/z 466.3 ($M+H^+$), 388.2 (M+H+-tBu).

EXAMPLE 4

[0082] This example illustrates the deprotection of (6*RS*)-6-isobutyl-5-((1*S*)-1-*t*-butoxycarbonyl-2-methylpropylamino)-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic acid allyl ester (**7**), followed by separation of the product into L,L- and D,L-diastereomers. The L,L-diastereomer is then subjected to a series of amino acid couplings. As noted in Example 1, the dihydropyrazinone unit is denoted by the three-letter code for the amino acid bearing the same side chain, the code being presented in brackets to indicate that it refers to the dihydropyrazinone unit rather than the amino acid. Bracketed three-letter codes that are not preceded by a superscript denote the (*S*)-stereoisomer of the unit. The dihydropyrazinone unit in this example is thus abbreviated as [Leu]. Bracketed three-letter codes that are

preceded by a superscript D, which appear in Example 5, denote the (*R*)-stereoisomer of the unit.

4.A. N-((3*S*)-3-Isobutyl-6-oxo-3,4,5,6-tetrahydropyrazin-2-yl)-L-Valine t-Butyl Ester (10, 10D) From (6*S*)-6-Isobutyl-5-((1*S*)-1-*t*-butoxycarbonyl-2-methylpropylamino)-3-oxo-3,6-dihydro-2*H*-pyrazine-1-carboxylic acid Allyl Ester (7)

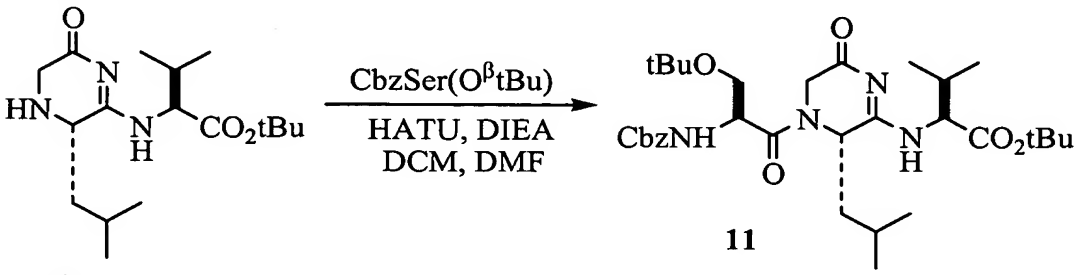


[0083] To 0.87 g (2.1 mmol) of **7** in 10 mL dry THF was added 10 mL of diethylamine (97 mmol), followed by $Pd(PPh_3)_4$ (0.48 g, 0.4 mmol). The reaction solution was stirred under argon at room temperature for 3 h. After concentration of the reaction mixture, the crude product was purified by flash chromatography to separate the diastereomers (1:99:0.5→ 2:98:0.5→ 3:97:0.5 EtOH/EtOAc/TEA). The overall yield of the reaction was 69%.

[0084] L,L-Diastereomer (**10**): R_f = 0.32 (UV, EtOH/EtOAc/TEA 10:90:1); ^{1}H NMR (400 MHz, CD_3OD) δ 0.98-1.04 (m, 12), 1.39 (m, 1, J = 5.0, 9.6, 14.3), 1.49 (s, 9), 1.72 (m, 1, J = 4.6, 10.7, 15.3), 1.70-1.80 (m, 1), 2.12 (m, 1, J = 6.6), 3.36 (d, J = 18.3), 3.44 (d, J = 18.3), 3.69 (dd, J = 5.0, 10.8), 4.65 (d, J = 5.9); MS (ES) m/z 326.3 ($M+H^+$), 270.3 ($M+H^+$-tBu).

[0085] D,L-Diastereomer (**10D**): R_f = 0.45 (UV, EtOH/EtOAc/TEA 10:90:1); ^{1}H NMR (400 MHz, CD_3OD) δ 0.83-0.92 (m, 12), 1.20 (m, 1, J = 5.1, 9.2, 14.1), 1.38 (s, 9), 1.63 (m, 1, J = 4.6, 10.6, 13.8), 1.75-1.85 (m, 1), 2.12 (m, 1, J = 6.7), 3.25 (d, 1, J = 18.4), 3.32 (d, 1, J = 18.4), 3.61 (dd, 1, J = 5.1, 10.8), 4.50(d, 1, J = 6.1); MS (ES) m/z 326.3 ($M+H^+$), 270.3 ($M+H^+$-tBu).

4.B. Cbz-Ser(O$^{\beta}$tBu)-[Leu]-Val t-Butyl Ester (11) From N-((3*S*)-3-Isobutyl-6-oxo-3,4,5,6-tetrahydropyrazin-2-yl)-L-Valine t-Butyl Ester (10)



10 **11**

Cbz-Ser(O$^{\beta}$tBu)-[Leu]-Val-OtBu

[0086] To 125 mg (0.36 mmol) of **10** in 15 mL of dry DCM and 2 mL of dry DMF was added DIEA (1.5 equiv), Cbz-Ser(tBu)-OH (1.5 equiv), and HATU (1.5 equiv). The reaction solution was stirred under argon at room temperature for 5 h. After concentration of the reaction mixture, the crude product was purified by flash chromatography (1:2 → 1:1 EtOAc/hexanes) to afford **11** as a white solid (75% yield, 9/1 ratio of *S/R* stereoisomers of the dihydropyrazinone unit). R_f = 0.43 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 27.0 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.85-0.94 (m, 12), 0.97 (s, 9), 1.30 (m, 1, J = 5.6, 10.5, 13.6), 1.40 (s, 9), 1.48-1.56 (m, 1), 1.61 (m, 1, J = 5.5, 14.8), 2.13 (m, 1, J = 6.6), 3.37-3.44 (m, 2), 3.84 (d, 1, J = 17.5), 4.36 (dd, 1, J = 5.9, 7.9), 4.46 (d, 1, J = 17.6), 4.51 (dd, 1, J = 8.0, 14.5), 4.97 (d, 1, J = 12.7), 5.07 (d, 1, J = 12.7), 5.44 (dd, 1, J = 3.8, 11.2), 7.29-7.36 (m, 5), 7.52 (d, 1, J = 7.7), 8.86 (d, 1, J = 7.9); MS (ES) m/z 603.5 ($M+H^+$); HRMS (FAB) m/z 603.3756 ($M+H^+$ $C_{32}H_{51}N_4O_7$ requires 603.3758).

4.C. Cbz-Glu(O$^{\gamma}$tBu)-Ser(O$^{\beta}$tBu)-[Leu]-Val t-Butyl Ester (12) From Cbz-Ser(O$^{\beta}$tBu)-[Leu]-Val t-Butyl Ester (11)

[0087] To 0.16 g (0.27 mmol) of **11** in 15 mL of methanol under an atmosphere of hydrogen was added 0.03 g (0.03 mmol) of 10% wt Pd/C. The reaction solution was stirred under an atmosphere of hydrogen for 1 h, after which the solution was filtered twice through Celite and concentrated. The crude product was dissolved in 15 mL of dry DCM and 2 mL of dry DMF, and to the reaction solution was added DIEA (1.5 equiv), Cbz-Glu(OtBu)-OH (1.5 equiv), and HATU (1.5 equiv). The reaction solution was stirred under nitrogen at room temperature overnight, then the mixture was concentrated and the crude product was redissolved in EtOAc. The organic layer was washed with NaH_2PO_4 (1 × 15 mL), saturated $NaHCO_3$ (1 × 15 mL), and brine (1 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 1:1 → 2:1 → 3:1) to afford **12** as a white solid (74% yield over two steps, 9/1 ratio of *S/R* stereoisomers of the

dihydropyrazinone unit). R_f = 0.18 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 28.5 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.84-0.94 (m, 12), 0.98 (s, 9), 1.30 (m, 1, J = 10.0), 1.37 (s, 9), 1.40 (s, 9), 1.45-1.53 (m, 1), 1.60 (m, 1, J = 14.4), 1.65-1.72 (m, 1), 1.74-1.83 (m, 1), 2.14 (m, 1, J = 6.5), 2.21 (m, 1), 3.42 (m, 2), 3.84 (d, 1, J = 17.5), 4.03 (dd, 1, J = 7.8), 4.38 (dd, 1, J = 6.2), 4.45 (d, 1, J = 17.6), 4.70 (dd, 1, J = 7.0), 4.98 (d, 1, J = 12.5), 5.02 (d, 1, J = 12.6), 5.44 (dd, 1, J = 3.2, 11.5), 7.28-7.38 (m, 5), 7.46 (d, 1, J = 7.9), 8.23 (d, 1, J = 7.3), 8.88 (d, 1, J = 7.9); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 18.6, 19.2, 22.0, 24.7, 27.2, 28.0, 28.1, 30.1, 31.6, 45.3, 46.8, 49.3, 53.8, 59.3, 62.3, 65.8, 73.3, 79.9, 81.5, 128.1, 128.2, 128.7, 137.4, 156.2, 169.7, 170.1, 171.5, 172.1, 173.1, 175.9; MS (ES) m/z 788.5 ($M+H^+$); HRMS (FAB) m/z 788.4795 ($M+H^+$ $C_{41}H_{66}N_5O_{10}$ requires 788.4810).

4.D. Cbz-Lys(N^{ϵ}Boc)-Glu(O^{γ}tBu)-Ser(O^{β}tBu)-[Leu]-Val t-Butyl Ester (13) From Cbz-Glu(O^{γ}tBu)-Ser(O^{β}tBu)-[Leu]-Val t-Butyl Ester (12)

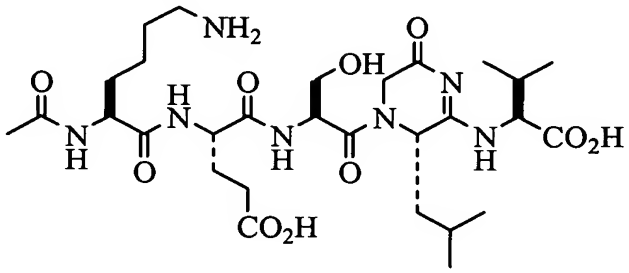
[0088] To 0.14 g (0.18 mmol) of **12** in 15 mL of methanol under an atmosphere of hydrogen was added 0.02 g (0.02 mmol) of 10% wt Pd/C. The reaction solution was stirred under an atmosphere of hydrogen for 3 h, after which the solution was filtered through Celite and concentrated. The crude product was dissolved in 15 mL of dry DCM and 2 mL of dry DMF, and to the reaction solution was added DIEA (1.5 equiv), Cbz-Glu(OtBu)-OH (1.5 equiv), and HATU (1.5 equiv). The reaction solution was stirred under nitrogen at room temperature overnight, then the mixture was concentrated and the crude product was redissolved in EtOAc. The organic layer was washed with NaH_2PO_4 (1 × 15 mL), saturated $NaHCO_3$ (1 × 15 mL), and brine (1 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 2:1 → 3:1 → 4:1) to afford **13** as a white solid (86% yield over two steps, 9/1 ratio of *S/R* stereoisomers of the dihydropyrazinone unit). R_f = 0.10 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 29.4 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$, major rotamer 10:1) δ 0.84-0.85 (d, 3, J = 6.6), 0.89 (d, 3, J = 6.4), 0.90 (d, 3, J = 6.8), 0.94 (d, 3, J = 6.9), 0.98 (s, 9), 1.25-1.35 (m, 4), 1.36 (s, 9), 1.38 (s, 9), 1.41 (s, 9), 1.44-1.52 (m, 2), 1.52-1.64 (m, 2, J = 4.0), 1.64-1.72 (m, 1), 1.75-1.84 (m, 1), 2.14 (m, 1, J = 6.6), 2.19 (t, 2, J = 8.4), 2.87 (m, 2, J = 6.2), 3.37-3.44 (m, 2), 3.84 (d, 1, J = 17.5), 3.94-3.99 (m, 1), 4.30 (dd, 1, J = 7.5, 13.6), 4.38 (dd, 1, J = 5.9, 7.9), 4.63 (d, 1, J = 17.7), 4.71 (dd, 1, J = 8.0), 5.01 (s, 2), 5.45 (dd, 1, J = 3.7, 11.6), 6.76 (dd, 1, J = 4.9, 6.0), 7.29-7.38 (m, 5), 7.40 (d, 1, J = 8.1), 7.95 (d, 1, J = 7.9), 8.29 (d, 1, J = 7.4), 8.88 (d, 1, J = 8.0); (minor rotamer, representative peaks) δ 7.01 (d, 1, J = 6.6), 8.02 (d, 1, J = 7.9), 8.08 (d, 1, J = 8.2), 9.11 (d, 1, J = 8.2); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 18.2, 18.9,

21.6, 22.9, 23.3, 24.3, 26.8, 27.6, 27.7, 28.3, 44.9, 46.4, 48.8, 51.1, 54.6, 58.9, 61.9, 65.4, 68.8, 73.0, 77.4, 79.6, 81.1, 127.7, 127.8, 128.4, 137.0, 155.6, 156.0, 169.3, 169.7, 170.7, 171.7, 171.9, 172.7, 174.5, 175.5, 183.7; MS (ES) m/z 1016.8 ($M+H^+$); HRMS (FAB) m/z 1016.6304 ($M+H^+$ $C_{52}H_{86}N_7O_{13}$ requires 1016.6284).

4.E. Ac-Lys(N^{ϵ}Boc)-Glu(O^{γ}tBu)-Ser(O^{β}tBu)-[Leu]-Val t-Butyl Ester (14) From Cbz-Lys(N^{ϵ}Boc)-Glu(O^{γ}tBu)-Ser(O^{β}tBu)-[Leu]-Val t-Butyl Ester (13)

[0089] To 60 mg (0.06 mmol) of **13** in 5 mL of methanol under an atmosphere of hydrogen was added 8 mg (0.007 mmol) of 10% wt Pd/C. The reaction solution was stirred under an atmosphere of hydrogen for 2 h, after which the solution was filtered through Celite and concentrated. The crude product was dissolved in 5 mL of dry DCM, and to the reaction solution was added DIEA (100 equiv) and acetic anhydride (100 equiv). The reaction solution was stirred under nitrogen at room temperature for 2 h, then the mixture was concentrated and the crude product was redissolved in DCM. The organic layer was washed with saturated $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product **14** was clean except for contamination by DIEA and was used without further purification (quantitative yield over two steps, 9/1 ratio of *S/R* stereoisomers of the dihydropyrazinone unit). R_f = 0.44 (UV, EtOH/EtOAc/TEA 10:90:1); retention time (analytical HPLC): 26.3 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.85 (d, 3, J = 6.6), 0.89 (d, 3, J = 6.4), 0.90 (d, 3, J = 6.8), 0.94 (d, 3, J = 6.8), 0.98 (s, 9), 1.18-1.52 (m, 33), 1.52-1.72 (m, 4), 1.77-1.84 (m, 1), 1.83 (s, 3), 2.10-2.19 (m, 3), 2.83-2.90 (m, 2), 3.36-3.42 (m, 2), 3.84 (d, 1, J = 17.5), 4.19 (dd, 1, J = 8.4, 13.5), 4.27 (dd, 1, J = 7.6, 13.6), 4.37 (dd, 1, J = 5.8, 8.0), 4.45 (d, 1, J = 17.7), 4.70 (dd, 1, J = 8.2, 14.0), 5.45 (dd, 1, J = 4.1, 11.6), 6.73 (t, 1, J = 5.2), 7.94 (d, 1, J = 5.8), 7.96 (d, 1, J = 7.6), 8.17 (d, 1, J = 7.5), 8.88 (d, 1, J = 7.9); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 12.3, 16.7, 18.0, 18.9, 21.1, 21.6, 22.6, 23.4, 23.7, 24.3, 26.6, 27.7, 27.8, 28.3, 29.8, 31.1, 41.7, 48.9, 51.2, 52.6, 53.4, 55.0, 59.0, 73.0, 77.4, 79.7, 81.2, 153.8, 155.6, 169.3, 169.4, 169.7, 170.7, 171.8, 171.9, 172.1, 172.7, 175.6; MS (ES) m/z 924.8 ($M+H^+$); HRMS (FAB) m/z 924.6048 ($M+H^+$ $C_{46}H_{82}N_7O_{12}$ requires 924.6021).

4.F. Ac-Lys-Glu-Ser-[Leu]-Val (15) From Ac-Lys(N^{ϵ}Boc)-Glu(O^{γ}tBu)-Ser(O^{β}tBu)-[Leu]-Val t-Butyl Ester (14)



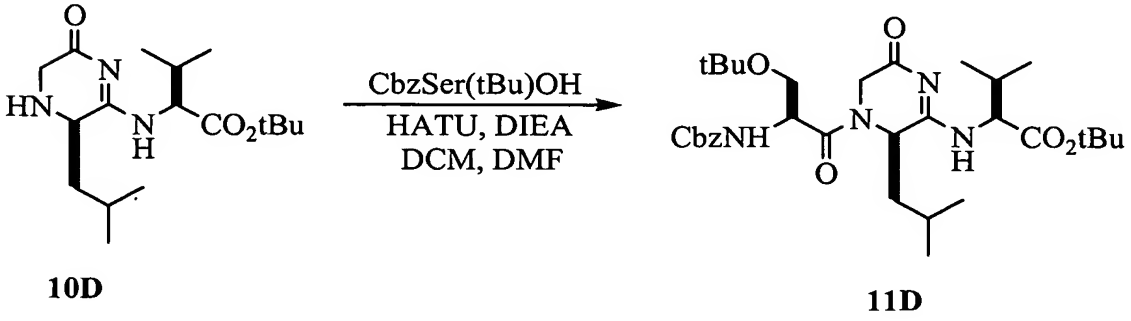
15

[0090] To 24 mg (0.03 mmol) of **14** was added 2.5 mL of trifluoroacetic acid and 1 drop of acetic acid. The reaction solution was warmed and stirred under nitrogen for 6 h. Most of the trifluoroacetic acid was removed by evaporation from dichloroethane (3 × 10 mL), then saturated $NaHCO_3$ was added to dissolve the crude product in a basic aqueous solution. The crude sample was filtered before purification by preparatory HPLC (Method B). HPLC fractions containing the pure product were frozen at –78°C before lyophilization, to yield **15** as a white solid (66% yield, single (*S*) diastereomer of the dihydropyrazinone unit). Retention time (analytical HPLC): 11.6 min; ^{1}H NMR (500 MHz, D_2O) δ 0.92 (d, 6, J = 5.5), 0.98-1.06 (m, 6), 1.40-1.60 (m, 4), 1.65-1.75 (m, 3), 1.75-1.85 (m, 1), 1.90-2.00 (m, 2), 2.02 (s, 3), 2.00-2.10 (m, 1), 2.28-2.35 (m, 1, J = 7.5), 2.40-2.50 (m, 2), 2.98 (t, 2, J = 7.5), 3.80-3.85 (m, 2), 4.25 (dd, 1), 4.30 (d, 1, J = 18.6), 4.38 (dd, 1), 4.51 (d, 1, J = 5.8), 4.73-4.80 (m, 1), 4.90 (t, 1, J = 7.2), 5.69 (dd, 1, J = 4.2, 11.6); MS (ES) m/z 656.5 ($M+H^+$); HRMS (FAB) m/z 656.3636 ($M+H^+$ $C_{29}H_{50}N_7O_{10}$ requires 656.3619).

EXAMPLE 5

[0091] This example illustrates the generation of a peptide analog identical to that of Example 4 except that the [Leu] unit is replaced by a [^{D}Leu] unit, the superscript D denoting the (*R*)-stereoisomer. The synthesis begins with the D,L-diastereomer **10D** of Section 4.A of Example 4.

5.A. Cbz-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (11D) From N-((3*S*)-3-Isobutyl-6-oxo-3,4,5,6-tetrahydropyrazin-2-yl)-L-Valine t-Butyl Ester (10D)



10D **11D**

[0092] Following the procedure described in Section 4.B above, the product was obtained at 64% yield and 1/20 ratio of *S/R* stereoisomers. R_f = 0.43 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 27.6 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.84-0.97 (m, 12), 1.09 (s, 9), 1.25-1.27 (m, 1), 1.37 (s, 9), 1.55-1.65 (m, 2), 2.12 (m, 1, J = 6.8), 3.40-3.50 (m, 2), 3.90 (d, 1, J = 17.6), 4.40 (dd, 1, J = 7.2), 4.49 (d, 1, J = 17.6), 4.53-4.58 (m, 1), 4.97 (d, 1, J = 11.3), 5.02 (d, 1, J = 12.4), 5.49 (dd, 1, J = 5.2, 11.8), 7.28-7.37 (m, 5), 7.47 (d, 1, J = 7.9), 9.09 (d, 1, J = 7.9); MS (ES) m/z 603.5 ($M+H^+$); HRMS (FAB) m/z 603.3763 ($M+H^+$ $C_{32}H_{51}N_4O_7$ requires 603.3758).

5.B. Cbz-Glu(O$^{\gamma}$tBu)-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (12D) From Cbz-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (11D)

[0093] Following the procedure described in Section 4.C above, the product was obtained with 75% yield over two steps, and a 1/20 ratio of *S/R* stereoisomers. R_f = 0.18 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 29.2 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$, major rotamer 7:2) δ 0.83-0.94 (m, 12), 1.10 (s, 9), 1.21-1.26 (m, 1), 1.37 (s, 9), 1.39 (s, 9), 1.56-1.88 (m, 4), 2.13 (m, 1, J = 6.5), 2.20 (dd, 2, J = 7.0), 3.47 (d, 2, J = 3.5), 3.90 (d, 1, J = 17.8), 3.98-4.06 (m, 1), 4.42-4.47 (m, 2), 4.76-4.80 (m, 1), 5.00 (d, 1, J = 12.3), 5.03 (d, 1, J = 12.3), 5.51 (dd, 1, J = 4.0, 11.5), 7.30-7.38 (m, 5), 7.47 (d, 1, J = 8.3), 8.03 (d, 1, J = 7.4), 9.09 (d, 1, J = 8.2); (minor rotamer, representative peaks) δ 3.61 (d, 1, J = 18.4), 4.13-4.18 (m, 1), 4.51 (d, 1, J = 18.3), 4.66 (dd, 1, J = 4.5), 5.10 (dd, 1, J = 4.2, 10.9), 7.96 (d, 1, J = 8.7), 9.20 (d, 1, J = 8.7); MS (ES) m/z 788.5 ($M+H^+$); HRMS (FAB) m/z 788.4824 ($M+H^+$ $C_{41}H_{66}N_5O_{10}$ requires 788.4810).

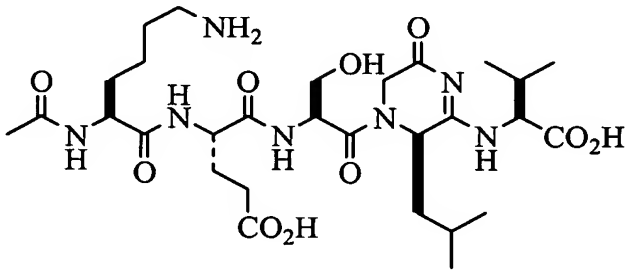
5.C. Cbz-Lys(N$^{\epsilon}$Boc)-Glu(O$^{\gamma}$tBu)-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (13D) From Cbz-Glu(O$^{\gamma}$tBu)-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (12D)

[0094] Following the procedure described in Section 4.D above, the product was obtained with 75% yield over two steps, and a 1/20 ratio of *S/R* stereoisomers. $R_f = 0.10$ (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 30.3 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$, major rotamer 2:1) δ 0.84-0.94 (m, 12), 1.09 (s, 9), 1.20-1.30 (m, 5), 1.30-1.43 (m, 27), 1.45-1.50 (m, 1), 1.50-1.70 (m, 4), 1.80-1.90 (m, 1), 2.10-2.25 (m, 3), 2.80-2.90 (m, 2), 3.45 (d, 2, J = 5.9), 3.90 (d, 1, J = 17.8), 3.90-4.00 (m, 1), 4.30 (dd, 1, J = 8.5, 13.6), 4.40-4.50 (m, 2), 4.77 (dd, 1, J = 7.6, 14.5), 5.02 (s, 2), 5.50 (dd, 1, J = 4.0, 11.2), 6.73 (t, 1, J = 6.0), 7.25-7.38 (m, 6), 7.95 (d, 1, J = 8.1), 8.06 (d, 1, J = 7.3), 9.09 (d, 1, J = 8.2); (minor rotamer, representative peaks) δ 1.70-1.80 (m, 1), 3.25-3.35 (m, 2), 3.60 (d, 1, J = 18.4), 4.35-4.40 (m, 1), 4.50 (d, 1, J = 18.4), 4.65 (dd, 1, J = 4.9, 8.7), 4.95-5.05 (m, 1), 5.10 (dd, 1, J = 11.3), 7.93 (d, 1, J = 8.8), 8.01 (d, 1, J = 10.0), 9.16 (d, 1, J = 8.7); MS (ES) m/z 1016.8 ($M+H^+$); HRMS (FAB) m/z 1016.6284 ($M+H^+$ $C_{52}H_{86}N_7O_{13}$ requires 1016.6284).

5.D. Ac-Lys(N$^{\epsilon}$Boc)-Glu(O$^{\gamma}$tBu)-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (14D) From Cbz-Lys(N$^{\epsilon}$Boc)-Glu(O$^{\gamma}$tBu)-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (13D)

[0095] Following the procedure described in Section 4.E above, the product was obtained in quantitative yield over two steps, and a 1/20 ration of *S/R* stereoisomers. $R_f = 0.44$ (UV, EtOH/EtOAc/TEA 10:90:1); retention time (analytical HPLC): 27.1 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$, major rotamer 3:1) δ 0.84-0.94 (m, 12), 1.09 (s, 9), 1.15-1.27 (m, 5), 1.30-1.42 (m, 2), 1.36 (s, 9), 1.37 (s, 9), 1.39 (s, 9), 1.52-1.70 (m, 3), 1.79-1.88 (m, 1), 1.83 (s, 3), 2,07-2.19 (m, 3), 2.83-2.91 (m, 2), 3.45 (d, 2, J = 5.9), 3.89 (d, 1, J = 17.8), 4.16-4.22 (m, 1), 4.26 (dd, 1, J = 8.2, 12.8), 4.43 (dd, 1, J = 5.8, 8.2), 4.44 (d, 1, J = 17.6), 4.76 (dd, 1, J = 6.0, 13.4), 5.49 (dd, 1, J = 4.0, 11.3), 6.76 (dd, 1, J = 5.6, 11.3), 7.96-8.01 (m, 3), 9.10 (d, 1, J = 8.2); (minor rotamer, representative peaks) δ 1.95 (s, 3), 3.61 (d, 1, J = 18.3), 4.34 (dd, 1), 4.49 (d, 1, J = 18.5), 4.65 (dd, 1, J = 4.8, 8.8), 5.03 (dd, 1, J = 6.9, 14.4), 5.10 (dd, 1, J = 11.5), 7.90 (d, 1, J = 7.9), 8.05 (d, 1, J = 8.3), 9.19 (d, 1, J = 8.7); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 18.4, 19.2, 20.9, 22.0, 22.9, 23.2, 23.5, 23.7, 24.3, 27.2, 27.3, 28.0, 28.1, 28.7, 29.7, 30.5, 31.7, 32.0, 45.8, 47.0, 51.7, 52.9, 59.0, 62.1, 67.0, 73.3, 77.7, 80.1, 81.5, 81.9, 155.9, 169.4, 169.7, 170.0, 171.1, 172.1, 172.4, 173.1, 175.8; MS (ES) m/z 924.7 ($M+H^+$); HRMS (FAB) m/z 924.6011 ($M+H^+$ $C_{46}H_{82}N_7O_{12}$ requires 924.6021).

5.E. Ac-Lys-Glu-Ser-[^{D}Leu]-Val (15D) From Ac-Lys(N$^{\epsilon}$Boc)-Glu(O$^{\gamma}$tBu)-Ser(O$^{\beta}$tBu)-[^{D}Leu]-Val t-Butyl Ester (14D)



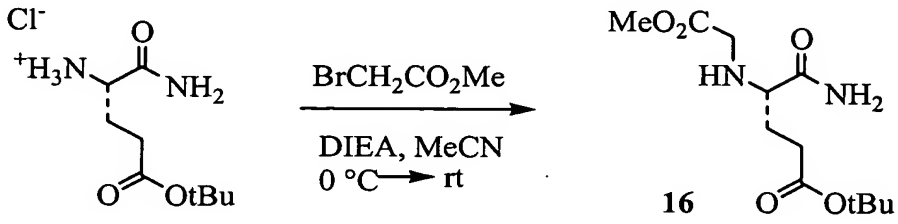
15D

Following the procedure of Section 4.F above, the product was obtained in 58% yield, with a single (*R*) stereoisomer. Retention time (analytical HPLC): 13.8 min; ^{1}H NMR (500 MHz, $(CD_3)_2SO$, rotamers) δ 0.84-0.89 (m, 12), 1.20-1.35 (m, 4), 1.40-1.70 (m, 5), 1.70-1.90 (m, 2), 1.82 (s, 1.8), 1.83 (s, 1.2), 2.00-2.20 (m, 3), 2.65-2.75 (m, 2), 3.85 (dd, 1, J = 3.4, 17.8), 3.87 (d, 1, J = 17.5), 4.13-4.25 (m, 3), 4.45 (d, 1, J = 17.8), 4.60 (m, 1), 4.60-4.70 (m, 1), 5.48 (dd, 1, J = 4.4, 10.7), 8.00-8.05 (m, 1), 8.11 (s, 1), 8.28 (d, 0.3, J = 8.0), 8.35 (d, 0.3, J = 8.0), 8.52 (s, 0.6); MS (ES) m/z 656.5 ($M+H^+$); HRMS (FAB) m/z 656.3636 ($M+H^+$ $C_{29}H_{50}N_7O_{10}$ requires 656.3619).

EXAMPLE 6

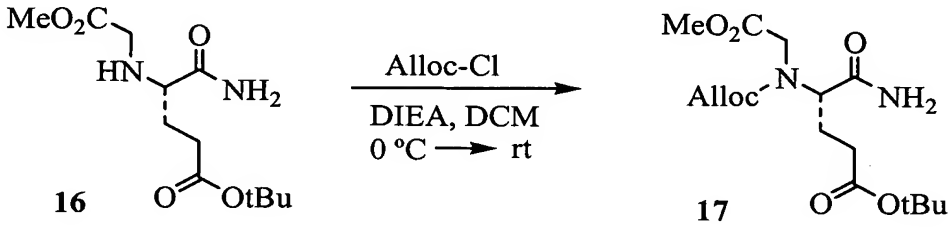
[0096] This example illustrates the liquid-phase synthesis of a peptide analog with protected side chains in which α-substituted dihydropyrazinones alternate with amino acid residues. As in the preceding examples, the dihydropyrazinone unit is denoted by a three-letter code in brackets, the three-letter code being that of the amino acid that bears the same side chain. The side chains on the dihydropyrazinone units in this peptide analog are those of leucine and the t-butyl ester of glutamic acid, and are thus abbreviated as [Leu] and [Glu(OtBu)]. The synthesis begins with reactions analogous to those of Example 1.

6.A. N-(2-Methoxy-2-oxoethyl)-L-Glutamic Acid, *t*-Butyl Ester Amide (16) from L-Glutamic Acid, *t*-Butyl Ester Amide Hydrochloride



[0097] To a solution of 1.0 g (4.2 mmol) of L-glutamic acid γ-*t*-butyl ester amide hydrochloride and 1.6 mL (9.2 mmol) of DIEA in 17 mL of dry MeCN at 0 °C was slowly added a solution of methyl bromoacetate (0.44 mL, 4.6 mmol) in dry MeCN (3 mL). The reaction mixture was stirred under argon and allowed to warm up to room temperature over a 15-hour period before it was diluted with 15 mL of saturated NH_4Cl. The aqueous layer was then made basic with 25 mL of saturated $NaHCO_3$ and the product was extracted with EtOAc (2 × 50 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford **16** as a white solid (0.92 g, 80% yield). $R_f = 0.41$ ($KMnO_4$/basic, EtOAc/MeOH/TEA 90:10:1); ^1H NMR (500 MHz, $CDCl_3$) δ 1.18 (s, 9), 1.64 (m, 1, J = 7.5), 1.72-1.76 (m, 1), 2.16 (t, 2, J = 7.5), 3.08 (d, 1, J = 17.5), 3.22 (d, 1, J = 17.5), 3.46 (s, 3), 6.77 (d, 1, J = 3.5), 7.07 (d, 1, J = 3.5); MS (ES) m/z 275.3 ($M+H^+$), 219.1 ($M+H^+$-tBu); HRMS (FAB) m/z 275.1608 ($M+H^+$ $C_{12}H_{23}N_2O_5$ requires 275.1607).

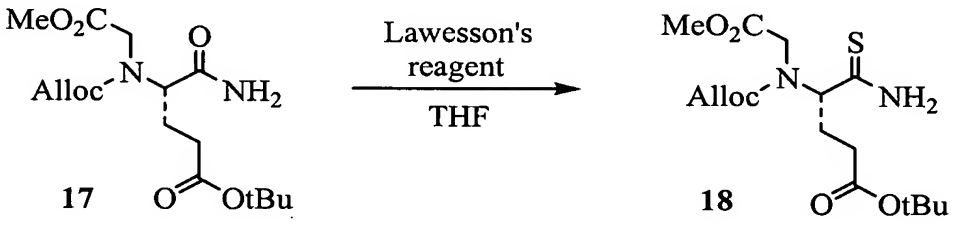
6.B. N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Glutamic Acid γ-*t*-Butyl Ester Amide (17) From N-(2-Methoxy-2-oxoethyl)-L-Glutamic Acid, *t*-Butyl Ester Amide (16)



[0098] To a solution of 0.70 g (2.6 mmol) of **16** and 1.8 mL (10.2 mmol) of DIEA in 20 mL of dry DCM at 0 °C was slowly added 0.81 mL (7.7 mmol) of allyl chloroformate. The reaction solution was stirred under argon and allowed to warm up to room temperature over a 15-hour period before it was diluted with 30 mL of saturated NH_4Cl. An additional 30 mL of DCM was added to the organic layer, which was further washed with saturated NH_4Cl (2 × 15 mL) and saturated $NaHCO_3$ (2 × 15 mL), then dried over Na_2SO_4, filtered, and

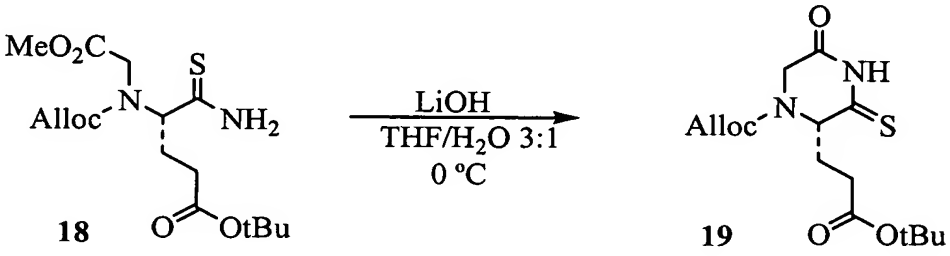
concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 1:2 → 1:1 → 2:1 → 3:1 → 4:1) to afford **17** as a yellow oil (0.73 g, 79% yield). $R_f = 0.35$ ($KMnO_4$/basic, EtOAc/hex 10:3); ^1H NMR (500 MHz, $CDCl_3$, rotamers) δ 1.23 (s, 9), 1.69-1.79 (m, 1), 2.00-2.20 (m, 3), 3.53 (s, 1.6) 3.57 (s, 1.4), 3.72 (d, 0.5, J = 17.7), 3.83 (d, 0.5, J = 18.0), 3.92 (d, 0.5, J = 17.2), 4.00-4.05 (m, 0.5), 4.08 (d, 0.5, J = 17.7), 4.34 (dd, 0.5, J = 5.8, 9.0), 4.35-4.42 (m, 2, J = 5.4), 4.97-5.13 (m, 2), 5.66 (m, 1, J = 5.3), 6.40 (s, 0.5), 6.43 (s, 0.5), 7.12 (s, 0.5), 7.55 (s, 0.5); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ 20.4, 23.8, 24.4, 27.5, 27.5, 31.3, 31.4, 46.3, 51.8, 52.0, 59.8, 66.1, 66.4, 79.8, 80.0, 116.9, 117.5, 131.5, 131.7, 155.0, 155.1, 170.5, 170.6, 171.7, 171.8, 172.3, 172.5; MS (ES) m/z 381.2 ($M+Na^+$), 258.1 ($M+Na^+$-tBu-$CONH_2$); HRMS (FAB) m/z 359.1819 ($M+H^+$ $C_{16}H_{27}N_2O_7$ requires 359.1818).

6.C. N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Glutamic Acid γ-*t*-Butyl Ester Thioamide (18) From N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Glutamic Acid γ-*t*-Butyl Ester Amide (17)



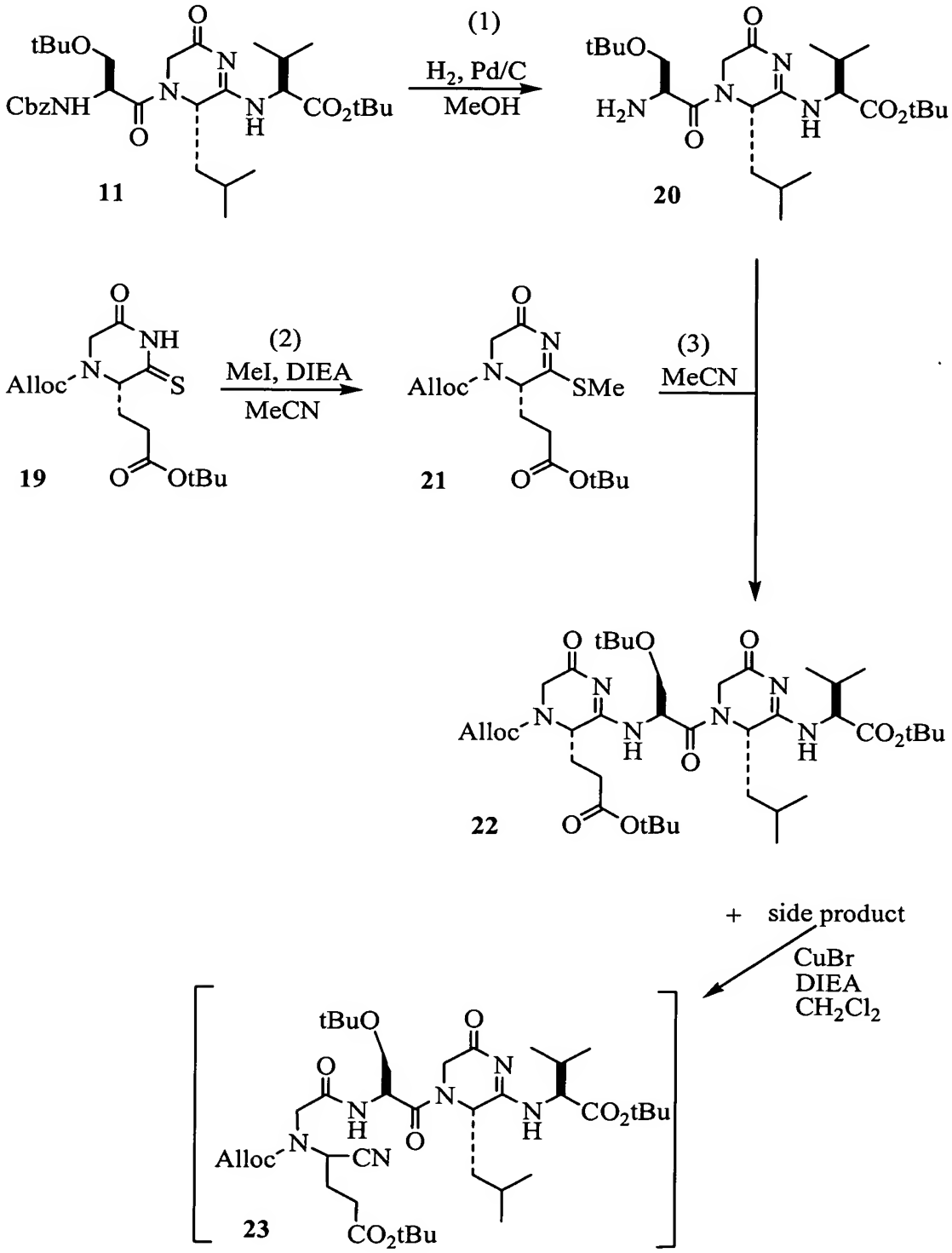
[0099] To 0.6 g (1.7 mmol) of **17** in 20 mL of dry THF was added 0.34 g (0.8 mmol) of Lawesson's reagent in two portions. The reaction solution was stirred under nitrogen, initially at 0 °C then with warming to room temperature. The reaction was stopped at 6 hours when no more starting material was observed by thin layer chromatography. After concentration of the reaction mixture, the crude product was purified by flash chromatography (EtOAc/hexanes 1:3 → 1:2 → 2:3) to afford **18** as a white solid (0.50 g, 80% yield). $R_f = 0.49$ (UV, $KMnO_4$, EtOAc/hexanes 1:1); ^1H NMR (500 MHz, $CDCl_3$, rotamers) δ 1.45 (s, 9), 2.02-2.09 (m, 1), 2.29-2.35 (m, 2), 2.60-2.70 (m, 0.6), 2.90-3.00 (m, 0.4), 3.75 (s, 1.8), 3.81 (s, 1.2), 3.98 (d, 1, J = 18.0), 4.14 (d, 1, J = 18.0), 4.60 (d, 1, J = 5.5), 4.63 (d, 1, J = 5.5), 4.60-4.65 (m, 0.4), 4.83 (dd, 0.6, J = 6.5), 5.21-5.35 (m, 2), 5.85-5.90 (m, 1), 7.79 (s, 0.5), 7.84 (s, 0.4), 8.62 (s, 0.5), 9.38 (s, 0.4); ^{13}C NMR (125 MHz, $CDCl_3$, rotamers) δ 26.7, 27.8, 28.0, 32.0, 32.3, 46.2, 52.5, 52.9, 63.7, 65.7, 66.9, 67.2, 80.7, 80.9, 117.8, 118.5, 131.6, 131.9, 155.6, 156.2, 171.0, 171.9, 172.4, 205.8, 206.3; MS (ES) m/z 397.2 ($M+Na^+$), 319.2 ($M+H^+$-tBu); HRMS (FAB) m/z 375.1583 ($M+H^+$ $C_{16}H_{27}N_2O_6S$ requires 375.1590).

6.D. N-Allyloxycarbonyl-2-(S)-(3-*t*-butoxy-3-oxopropyl)-5-oxo-3-thionopiperazine (19) From N-Allyloxycarbonyl-N-(2-methoxy-2-oxoethyl)-L-Glutamic Acid γ-t-Butyl Ester Thioamide (18)



[0100] To 0.29 g (0.78 mmol) of **18** in 20 mL of THF/H_2O 3:1 at 0 °C was added 1 M LiOH (0.78 mL, 0.78 mmol), which turned the solution bright yellow. The reaction solution was stirred under nitrogen and quenched after 15 minutes by addition of solid $KHSO_4$. After concentration of the reaction mixture, the aqueous solution was extracted with EtOAc (2 × 30 mL). The organic layer was further washed with 1 M $KHSO_4$ (1 × 15 mL), saturated $NaHCO_3$ (1 × 15 mL), and brine (1 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexanes 1:3) to afford **19** as a yellow oil (0.22 g, 83% yield, >99% e.e.). R_f = 0.58 (UV, EtOAc/hexanes 1:1); retention time (analytical HPLC): 24.8 min; retention time (chiral HPLC): 8.3 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (s, 9), 2.10-2.17 (m, 1), 2.23-2.36 (m, 3), 3.80-4.10 (m, 1), 4.61 (s, 2), 4.80-5.00 (m, 1), 5.24 (dd, 1, J = 1.0, 10.5), 5.30 (dd, 1, J = 1.0, 17.0), 5.89 (m, 1, J = 5.5, 10.5, 16.0), 10.24 (s, 1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.6, 27.9, 31.7, 42.2, 42.7, 52.5, 61.8, 62.4, 67.2, 81.0, 81.1, 118.8, 131.7, 153.8, 156.6, 164.9, 168.6, 171.3, 171.8, 203.6, 205.1; MS (ES) m/z 365.2 ($M+Na^+$), 309.1 ($M+Na^+$-tBu).

6.E. Coupling Reactions Starting With N-Allyloxycarbonyl-2-(S)-(3-*t*-butoxy-3-oxopropyl)-5-oxo-3-thionopiperazine (19) and Cbz-Ser(O$^{\beta}$tBu)-[Leu]-Val t-Butyl Ester (11, From Example 4.B) to Form Alloc-[Glu(OtBu)]-Ser(tBu)-[Leu]-Val-*t*-Butyl Ester (22)

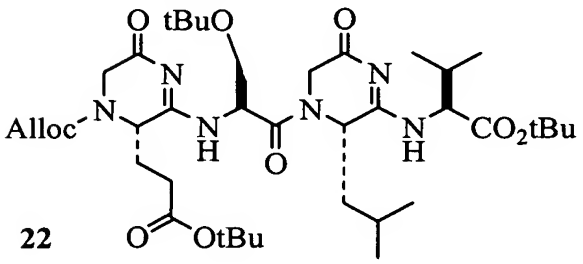


[0101] Reaction (1): To 0.20 g (0.34 mmol) of **11** in 30 mL of MeOH under an atmosphere of argon was added 36 mg (0.03 mmol) of 10% wt Pd/C. The argon gas was removed with vacuum and replaced with an atmosphere of hydrogen. Hydrogen gas was bubbled through the stirred reaction solution for 30 min, after which the solution was filtered through Celite and concentrated. The crude product, **20**, was used without further purification.

[0102] Reaction (2): To 0.17 g (0.5 mmol) of **19** in 5 mL of MeCN under an atmosphere of argon at 0 °C was added 0.19 mL (3.0 mmol) of methyl iodide followed by 88 μL (0.5 mmol) of DIEA. The reaction solution was then sealed and stirred for 1 hour. When no more starting material was observed, the reaction was stopped and the solvent and excess methyl iodide were removed by rotary evaporation to give the acyl thioimidate **21**.

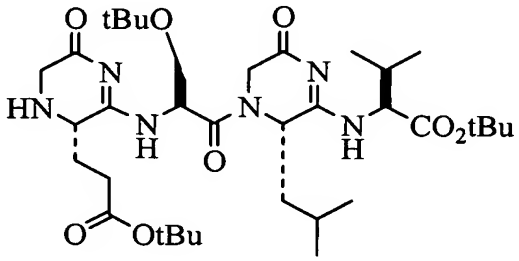
[0103] Reaction (3) The crude amine **20** was dissolved in 5 mL of MeCN and added to excess acyl thioimidate **21**. The reaction solution was stirred under nitrogen at room temperature for 20 hours. The reaction mixture was then concentrated and the crude product was purified by flash chromatography (EtOAc/hexanes 1:1 → 2:1 → 3:1 → 5:1 → EtOAc) to afford a mixture of the desired product **22** and a side product. The side product was converted to the nitrile **23** upon addition of CuBr and DIEA in DCM, and then separated easily from the desired product, **22**, by flash chromatography (EtOAc/hexanes 1:1 → 3:1 → 6:1 → EtOAc0; **22** was obtained as a clear oil (94.4 mg, 36% yield over 3 steps). $R_f = 0.27$ (UV, EtOAc); ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.82 (d, 3, J = 6.5), 0.86 (d, 3, J = 6.5), 0.90 (d, 3, J = 7.0), 0.94 (d, 3, J = 7.0), 0.99 (s, 9), 1.25-1.40 (m, 1), 1.37 (s, 9), 1.41 (s, 9), 1.55-1.60 (m, 2), 1.75-1.80 (m, 2), 2.10-2.20 (m, 1), 2.20-2.30 (m, 2), 3.40-3.50 (m, 1), 3.55-3.60 (m, 1), 3.75 (d, 0.5), 3.85 (d, 0.5), 3.97 (d, 1, J = 17.5), 4.13 (d, 1, J = 18.5), 4.38-4.39 (m, 1), 4.42 (d, 1, J = 17.0), 4.55 (m, 2), 4.80-4.90 (m, 1), 4.90-5.00 (m, 1), 5.20-5.40 (m, 2), 5.44 (dd, 1, J = 3.5, 11.5), 5.85-6.00 (m, 1), 8.90 (d, 1, J = 8.0), 9.02 (d, 0.5), 9.05 (d, 0.5); MS (ES) m/z 777.6 ($M+H^+$); ^{13}C NMR (125 MHz, $(CD_3)_2SO$, rotamers) δ 18.1, 18.9, 21.4, 23.4, 24.2, 26.8, 27.6, 27.7, 29.7, 31.4, 45.0, 46.5, 59.2, 50.3, 58.9, 61.3, 65.8, 73.2, 79.9, 81.1, 117.2, 117.4, 132.9, 153.7, 168.9, 169.3, 170.1, 170.3, 171.2, 172.6, 175.1, 175.6; HRMS (FAB) m/z 777.4743 ($C_{39}H_{65}N_6O_{10}$ requires 777.4762).

6.F. Cbz-Lys(Boc)-[Glu(OtBu)]-Ser(tBu)-[Leu}-Val-*t*-Butyl Ester (25) From Alloc-[Glu(OtBu)]-Ser(tBu)-[Leu]-Val-*t*-Butyl Ester (22)



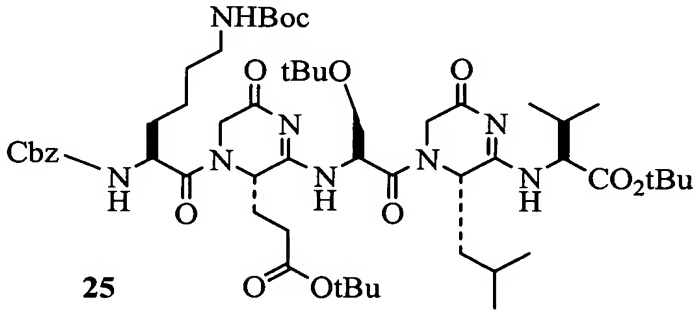
22

(1) $Pd(PPh_3)_4$, $HNEt_2$/THF 1:1



24

(2) Cbz-Lys(Boc), HATU, DIEA, DCM/DMF



25

[0104] Reaction (1): To 94 mg (0.12 mmol) of **22** in 2 mL of dry THF was added 2 mL of diethylamine, followed by $Pd(PPh_3)_4$ (28 mg, 0.02 mmol). The reaction solution was stirred under nitrogen at room temperature for 1 hour. After concentration of the reaction mixture, the crude amine **24** was purified by flash chromatography (EtOAc/hexanes/TEA 75:25:1 → EtOAc/TEA 100:1 → EtOH/EtOAc/TEA 5:95:1 → 10:90:1) to afford **24** as a yellow oil (78 mg, 92%). R_f = 0.09 (UV, EtOH/EtOAc 1:9); MS (ES) m/z 693.6 ($M+H^+$).

[0105] Reaction (2): To **24** in 5 mL of dry DCM and 1 mL of dry DMF was added 92 mg (0.24 mmol) of Cbz-Lys(Boc) followed by 43 μL (0.24 mmol) of DIEA and 92 mg (0.24 mmol) of HATU. The reaction solution was stirred under nitrogen at room temperature for 18 hours, then the mixture was concentrated and the crude product was redissolved in EtOAc. The organic layer was washed with NaH_2PO_4 (1 × 15 mL), saturated $NaHCO_3$ (1 × 15 mL), and brine (1 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was

purified by flash chromatography (EtOAc/hexanes 1:1 → 3:1 → EtOAc → EtOH/EtOAc 5/95) to afford **25** as a white solid (87 mg, 74% yield). R_f = 0.28 (UV, EtOAc); ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 0.82 (d, 3, J = 6.5), 0.87 (m, 3), 0.90 (d, 3, J = 7.0), 0.94 (d, 3, J = 7.0), 1.00 (s, 9), 1.20-1.50 (m, 3), 1.36 (s, 9), 1.37 (s, 9), 1.41 (s, 9), 1.50-1.65 (m, 6), 1.70-1.80 (m, 2), 2.10-2.20 (m, 2), 2.25-2.35 (m, 1), 2.80-2.95 (m, 2), 3.50-3.60 (m, 2), 3.90-4.10 (m, 2), 4.20-4.30 (m, 2), 4.35-4.50 (m, 2), 4.90-5.00 (m, 1), 5.02 (s, 2), 5.13 (dd, 1), 5.45 (dd, 1), 6.76 (t, 1), 7.30-7.35 (m, 5), 8.92 (d, 1, J = 8.0), 9.09 (d, 1, J = 6.5); MS (ES) m/z 1055 ($M+H^+$).

[0106] The foregoing is offered primarily for purposes of illustration. Further modifications and variations that still embody the underlying concepts of this invention and fall within its scope will be apparent to those skilled in the art.